

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**



PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | |
|--|---|---|
| <p>(51) International Patent Classification ⁶ : C07K 14/72, 16/28, G01N 33/74</p> | <p>A2</p> | <p>(11) International Publication Number: WO 95/16709 (43) International Publication Date: 22 June 1995 (22.06.95)</p> |
| <p>(21) International Application Number: PCT/US94/14643 (22) International Filing Date: 13 December 1994 (13.12.94) (30) Priority Data: 08/166,333 13 December 1993 (13.12.93) US 08/173,512 23 December 1993 (23.12.93) US (60) Parent Applications or Grants (63) Related by Continuation US 08/166,333 (CIP) Filed on 13 December 1993 (13.12.93) US 08/173,512 (CIP) Filed on 23 December 1993 (23.12.93) (71) Applicants (for all designated States except US): BIOGEN, INC. [US/US]; 14 Cambridge Center, Cambridge, MA 02142 (US). INSERM (U.293) [FR/FR]; 1, rue Maurice-Arnoux, F-29120 Montrouge (FR). (72) Inventors; and (75) Inventors/Applicants (for US only): CATE, Richard, L. [US/US]; 14 Cambridge Center, Cambridge, MA 02142 (US). JOSSO, Nathalie [FR/FR]; INSERM (U.293), 1, rue Maurice-Arnoux, F-29120 Montrouge (FR).</p> | <p>(74) Agents: HALEY, James, F., Jr. et al.; Fish & Neave, 1251 Avenue of the Americas, New York, NY 10020-1104 (US). (81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ). Published <i>Without international search report and to be republished upon receipt of that report.</i> <i>With an indication in relation to a deposited microorganism furnished under Rule 13^{bis} separately from the description.</i> <i>Date of receipt by the International Bureau:</i> 27 January 1995 (27.01.95)</p> | |
| <p>(54) Title: ANTI-MULLERIAN HORMONE RECEPTOR POLYPEPTIDES AND ANTIBODIES THERETO (57) Abstract This invention relates to polypeptides displaying the activity of anti-Mullerian hormone (AMH) receptor, also known as Mullerian inhibiting substance (MIS) receptors, and antibodies to those polypeptides. More particularly, this invention relates to such AMH receptor polypeptides and antibodies, processes for producing those polypeptides and antibodies and methods for using them in the treatment of cancer and tumors of tissues associated with expression of the anti-Mullerian hormone receptor.</p> | | |

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | |
|----|--------------------------|----|---------------------------------------|----|--------------------------|
| AT | Austria | GB | United Kingdom | MR | Mauritania |
| AU | Australia | GE | Georgia | MW | Malawi |
| BB | Barbados | GN | Guinea | NE | Niger |
| BE | Belgium | GR | Greece | NL | Netherlands |
| BF | Burkina Faso | HU | Hungary | NO | Norway |
| BG | Bulgaria | IE | Ireland | NZ | New Zealand |
| BJ | Benin | IT | Italy | PL | Poland |
| BR | Brazil | JP | Japan | PT | Portugal |
| BY | Belarus | KE | Kenya | RO | Romania |
| CA | Canada | KG | Kyrgyzstan | RU | Russian Federation |
| CF | Central African Republic | KP | Democratic People's Republic of Korea | SD | Sudan |
| CG | Congo | KR | Republic of Korea | SE | Sweden |
| CH | Switzerland | KZ | Kazakhstan | SI | Slovenia |
| CI | Côte d'Ivoire | LI | Liechtenstein | SK | Slovakia |
| CM | Cameroon | LK | Sri Lanka | SN | Senegal |
| CN | China | LU | Luxembourg | TD | Chad |
| CS | Czechoslovakia | LV | Latvia | TG | Togo |
| CZ | Czech Republic | MC | Monaco | TJ | Tajikistan |
| DE | Germany | MD | Republic of Moldova | TT | Trinidad and Tobago |
| DK | Denmark | MG | Madagascar | UA | Ukraine |
| ES | Spain | ML | Mali | US | United States of America |
| FI | Finland | MN | Mongolia | UZ | Uzbekistan |
| FR | France | | | VN | Viet Nam |
| GA | Gabon | | | | |

ANTI-MULLERIAN HORMONE RECEPTOR
POLYPEPTIDES AND ANTIBODIES THERETO

TECHNICAL FIELD OF INVENTION

This invention relates to polypeptides

- 5 displaying the activity of anti-Mullerian hormone
receptors and antibodies and antibody homologs to those
polypeptides. More particularly, this invention
relates to such polypeptides and antibodies, processes
for producing those polypeptides and antibodies and
10 methods for using them in the treatment of cancer and
tumors of tissues associated with expression of the
anti-Mullerian hormone receptor.

BACKGROUND OF INVENTION

- Anti-Mullerian hormone (AMH), also called
15 Mullerian inhibiting substance (MIS), is a glycoprotein
produced by prepubertal Sertoli cells and by postnatal
granulosa cells. It is a non-steroidal factor that
causes regression of the Mullerian duct, the anlage of
the internal female reproductive tract, in the male
20 fetus. AMH is secreted at low levels by postnatal
gonadal cells. The significance of postnatal AMH is
not fully understood.

- AMH has been hypothesized to be useful in
treating tumors that derive from the Mullerian duct
25 (e.g., uterus, Fallopian tubes) and tumors of tissues
that derive from progenitor cells of the Mullerian duct
(e.g., ovaries), by binding to the AMH receptor and

- 2 -

inhibiting cell proliferation. However, there have been problems identifying the functional AMH ligand. An alternate approach to the treatment of such tumors is to use the AMH receptor to target the tumors.

5 AMH is part of the transforming growth factor- β (TGF- β) superfamily. It is structurally and functionally related to TGF- β , bone morphogenic protein (BMP) and activin. Receptors have been isolated for some of these ligands, including the activin type II
10 receptor (ActR-II) (L.Mathews and W.Vale, Cell, 65, p. 973 (1993)) and the TGF- β type II receptor (TGFBR-II) (H.Lin et al. Cell, 68, p. 775 (1992)). More recently, investigators have identified a type I receptor which may be associated with ActR-II (L.Attisano et al.,
15 Cell, 75, pp. 671-680 (1993)) and/or with TGFBR-II (R.Ebner et al., Science, 260, pp. 1344-1348 (1993)). This same receptor has also been identified as an AMH (or Mullerian inhibiting substance) receptor by W.He et al., Developmental Dynamics, 196, pp. 133-142 (1993).
20 However, its expression profile is not at all consistent with the expected expression profile of an AMH receptor.

AMH receptors are present in a very limited number of body tissues. Therefore, the AMH receptor is
25 particularly useful for developing antibody-toxin conjugates to target tumor treatments. Antibody-toxin complexes targeted to the AMH receptor can be used much more aggressively than antibody-toxin complexes targeted to other receptors which are typically more
30 prevalent in the body.

SUMMARY OF INVENTION

This invention provides isolated DNA sequences encoding AMH receptor polypeptides. This invention further provides recombinant DNA molecules
35 comprising an AMH receptor DNA sequence and capable of

- 3 -

directing expression of an AMH receptor polypeptide. This invention also provides isolated AMH receptor polypeptides which possesses the biological or antigenic activity of native AMH receptor.

5 Another aspect of this invention provides a method for detecting the AMH receptor polypeptide in vitro or in vivo comprising the steps of contacting an AMH receptor antibody with a sample or cell suspected of containing the receptor polypeptide and detecting if
10 binding has occurred.

 This invention also provides an assay for detecting the presence of the AMH ligand in a test sample by contacting the test sample with the AMH receptor polypeptide and determining whether binding
15 has occurred.

 Another aspect of this invention provides a method for identifying and then isolating and purifying molecules that bind to an AMH receptor polypeptide comprising contacting a sample containing the test
20 molecules with an AMH receptor polypeptide immobilized on a support under conditions whereby the molecules to be identified are selectively adsorbed onto the immobilized receptor, washing the immobilized support to remove non-adsorbed material and separating the
25 bound molecule from the immobilized AMH receptor polypeptide to which they are adsorbed.

 This invention also provides antibodies and antibody homologs capable of binding to the AMH receptor polypeptide. Another aspect of this invention
30 provides a pharmaceutical composition comprising an antibody capable of binding to the AMH receptor polypeptide conjugated with a toxin or radionuclide capable of killing or preventing growth of a cell expressing the AMH receptor polypeptide. A further
35 aspect of this invention is a method of treating cancers or tumors in tissues characterized by the

- 4 -

expression of the AMH receptor polypeptide by administering an AMH receptor antibody-toxin or antibody-radionuclide conjugate.

BRIEF DESCRIPTION OF THE DRAWINGS

5 Figure 1A is a schematic diagram showing proteins encoded by three clones (7F2, 2B10 and 3D6) isolated from a fetal ovary library and by clone H1, constructed by joining the extracellular domain of 3D6 to the transmembrane kinase region of 2B10 using a
10 BspM1 restriction site. The domain encoded by an extra exon in 3D6 is hatched. The signal peptide is shown in black and introns A (159 bp) and B (120 bp) are represented by arrowheads.

 Figures 1B and 1C represent the nucleotide
15 sequence and translated amino acid sequence of clone H1. The BspM1 restriction site used for the construction is indicated. The nucleotide sequence of the extra exon found in 3D6 is shown in lower case and its translated protein sequence is underlined. The
20 transmembrane region is shaded and the predicted signal sequence cleavage sites for both the H1 and the 2B10 proteins are indicated by arrows. Introns A and B are indicated by arrowheads. Potential N-linked
25 glycosylation sites are boxed. Two sites at which nucleotide differences were observed between 7F2, 2B10 and 3D6 are also indicated. H1 contains the consensus sequence at these two positions.

 Figure 1D depicts a comparison of the rabbit
30 AMH receptor encoded by H1 with the human TGF- β type II receptor and the mouse activin type II receptor. Amino acids shared by at least two receptors are shaded. Cysteines conserved in all three extracellular domains are indicated by a dot. Roman numbers indicate protein kinase catalytic consensus domains. Domains VIB and
35 VIII are specific for serine-threonine kinases.

- 5 -

Figures 2A and 2B depict Northern blot hybridization of 7F2 to mRNA extracted from various organs of developing rabbits. "SC" represents Sertoli cells.

5 Figures 2C-2E depict in situ hybridization of rabbit reproductive tissues: (C) adult (16 weeks) ovary containing follicles at various developmental stages; (D) 18-day-old fetal testis; and (E) 18-day-old female fetal, sexually undifferentiated reproductive tract
10 containing Mullerian (M) and Wolffian (W) ducts.

 Figure 3 depicts (A) cells transfected with H1 and exposed to plasmin-cleaved AMH; (B) cells transfected with H1 and exposed to full-length AMH; (C) cells transfected with 2B10 and exposed to plasmin-
15 cleaved AMH; and (D) cells transfected with β -galactosidase DNA and exposed to plasmin-cleaved AMH. Negative COS cells are indicated by arrows. Dark field illumination, x 500.

 Figure 4A is a schematic diagram showing
20 primers used for reverse-transcriptase polymerase chain (RT-PCR) reaction and the expected PCR fragments generated for the two receptor isoforms, i.e., 164 bp for 2B10 and 347 bp for H1. The PCR oligonucleotides are indicated by arrows, the sense oligonucleotide is
25 5' GCAGGATGCT GGGCACTCTG 3' [SEQ ID NO: 8]
 and the antisense oligonucleotide is

 5' GTCAGCACCA CAGGAGCAGG 3' [SEQ ID NO: 9]

 Figure 4B depicts the gel analysis of RT-PCR products generated from RNAs extracted from various
30 rabbit organs.

 Figure 5 depicts a comparison of the rabbit (bottom line) [SEQ ID NO: 4] and human (top line) [SEQ ID NO: 13] AMH receptor protein sequences.

 Figure 6 represents a partial nucleotide
35 sequence of the AMH receptor gene of patient T.A. [nucleotides 401 to 800 of SEQ ID NO: 14]. The exon is

- 6 -

shown in upper case letters and the intron is shown in lower case letters. A G>A mutation is indicated by an arrow.

Figure 7 represents a partial nucleotide sequence [SEQ ID NO: 14] of the AMH receptor gene isolated from the λ EMBL4 library. Exons are in upper case; introns are in lower case. The nucleotide that is mutated in patient T.A. is indicated by an arrow. The protein sequence is shown below the exon sequences.

Figure 8 depicts an electrophoretic analysis of RT-PCR products generated with RNA isolated from control tissues from normal individuals and with RNA from the testis of patient T.A.. The expected band is observed in the control samples while two aberrant bands are seen in the T.A. sample. The smaller band represents an mRNA that has undergone exon skipping, while the other band, which is slightly larger than the normal PCR product in the control lanes, reflects an mRNA that has undergone cryptic splicing.

Figure 9 is a schematic diagram showing the generation of the two aberrant mRNAs in patient T.A., caused by the splicing mutation in the AMH receptor gene.

Figure 10 depicts an electrophoretic analysis of RT-PCR product generated with RNA from the four human granulosa cell tumors. Lane 1 -- ovarian tissue, patient 1; Lane 2 -- ovarian tissue, patient 2; Lane 3 -- ovarian tissue, patient 3; Lane 4 -- metastasis, patient 3; Lane 5 -- size marker PhiX-Hae III. The expected band is seen clearly in lanes 1 and 2 and more faintly in lanes 3 and 4, indicating that the tumors express the AMH receptor.

DETAILED DESCRIPTION OF THE INVENTION

An isolated AMH receptor DNA sequence is a DNA sequence that is identified and separated from at

- 7 -

least one contaminant DNA sequence with which it is ordinarily associated in the natural source of the AMH receptor. An isolated AMH receptor DNA sequence is other than in the form or setting in which it is found
5 in nature. An isolated AMH receptor DNA sequence therefore is distinguished from the AMH receptor DNA sequence as it exists in natural cells.

Preferably, the isolated AMH receptor DNA sequence comprises a DNA sequence encoding the mature
10 AMH receptor polypeptide selected from the group consisting of

- (a) nucleotides 113 to 1585 of SEQ ID NO: 1;
- (b) nucleotides 110 to 1765 of SEQ ID NO: 2;
- (c) nucleotides 112 to 1779 of SEQ ID NO: 12;
- 15 (d) DNA sequences that hybridize to any of the DNA sequences of (a)-(c) and which encode a biologically or antigenically active AMH receptor polypeptide; and
- (e) DNA sequences that are degenerate to any of
20 the foregoing DNA sequences.

Alternatively, the isolated AMH receptor DNA sequence comprises a DNA sequence encoding the extracellular domain of the AMH receptor polypeptide selected from the group consisting of

- 25 (a) nucleotides 113-310 of SEQ ID NO: 1;
- (b) nucleotides 110-490 of SEQ ID NO: 2;
- (c) nucleotides 112-492 of SEQ ID NO: 12;
- (d) DNA sequences that hybridize to any of the DNA sequences of (a)-(c) and which encode a
30 biologically or antigenically active AMH receptor polypeptide; and
- (e) DNA sequences that are degenerate to any of the foregoing DNA sequences.

"Hybridization" as used in this application
35 means hybridization carried out under conditions of high or moderate stringency. High stringency

- 8 -

conditions are defined as hybridizing with plaque screen buffer (0.2% polyvinylpyrrolidone, 0.2% Ficoll-400, 0.2% bovine serum albumin, 50mM Tris-HCl (pH 7.5), 1M NaCl, 0.1% sodium pyrophosphate, 1% SDS), 10%
5 dextran sulphate, and 100 µg/ml denatured, sonicated salmon sperm DNA at 65°C for 12-20 hours, and washing with 75 mM NaCl/7.5 mM sodium citrate (0.5 X SCC)/1% SDS at 65°C. Moderate stringency conditions are defined as hybridizing with plaque screen buffer, 10%
10 dextran sulphate and 100 µg/ml denatured, sonicated salmon sperm DNA at 55°C for 12-20 hours, and washing with 300 mM NaCl/30 mM sodium citrate (2.0 X SCC)/1% SDS at 55°C.

The isolated AMH receptor polypeptides of
15 this invention are polypeptides that possess the biological or antigenic activity of native AMH receptor. The biological activity of the native AMH receptor is the ability to bind to AMH ligand. The antigenic activity of the native AMH receptor is the
20 ability to raise antibody that binds with the receptor. The isolated AMH receptor polypeptides of this invention are separated from at least one contaminant polypeptide with which they are ordinarily associated in the natural source of the AMH receptor. An isolated
25 AMH receptor polypeptide is other than in the form or setting in which it is found in nature. An isolated AMH receptor polypeptide therefore is distinguished from the AMH receptor polypeptide as it exists in natural cells.

30 The AMH receptor polypeptides of this invention may exist in monomeric or oligomeric forms. Oligomeric forms may be composed of only the AMH polypeptide or may include other TGF-β superfamily type I receptors.

35 Preferably the AMH receptor polypeptides of this invention are selected from the group consisting

- 9 -

of polypeptides encoded by the isolated AMH receptor DNA sequences of this invention. More preferably, an AMH receptor polypeptide of this invention comprises an amino acid sequence corresponding to the mature AMH
5 receptor polypeptide selected from the group consisting of

- (a) amino acid residues 18 to 508 of SEQ ID NO: 3;
- (b) amino acid residues 18 to 569 of SEQ ID
10 NO: 4; and
- (c) amino acid residues 18 to 573 of SEQ ID NO: 13.

Alternatively, an isolated AMH receptor polypeptides of this invention comprises an amino acid
15 sequence corresponding to the extracellular domain the mature AMH receptor polypeptide selected from the group consisting of

- (a) amino acid residues 18 to 83 of SEQ ID NO: 3;
- (b) amino acid residues 18 to 144 of SEQ ID
20 NO: 4; and
- (c) amino acid residues 18 to 144 of SEQ ID NO: 13.

An antibody capable of binding to AMH receptor polypeptide is an antibody that binds to the
25 polypeptide and is identified and separated and/or recovered from a component of any natural environment in which it may be present. Preferably, such antibody is capable of binding to the extracellular domain of the AMH receptor polypeptide. More preferably, such
30 antibody is capable of binding to the AMH receptor in its native conformation on the surface of cells.

An antibody homolog is a protein comprising one or more polypeptides selected from immunoglobulin light chains, immunoglobulin heavy chains, and antigen-
35 binding fragments thereof, which are capable of binding to one or more antigens. The component polypeptides of

- 10 -

an antibody homolog composed of more than one polypeptide may optionally be disulfide-bound or otherwise covalently crosslinked. Accordingly, antibody homologs include intact immunoglobulins of types IgA, IgG, IgE, IgD, IgM (as well as subtypes thereof), wherein the light chains of the immunoglobulin may be of types kappa or lambda. Antibody homologs also include portions of intact immunoglobulins that retain antigen-binding specificity, for example, Fab fragments, Fab' fragments, F(ab')₂ fragments, F(v) fragments, heavy chain monomers or dimers, light chain monomers or dimers, dimers consisting of one heavy and one light chain, and the like.

Also encompassed in the term "antibody homologs" are humanized recombinant antibody homologs and chimeric recombinant antibody homologs. A humanized recombinant antibody homolog is an antibody homolog initially derived from a nonhuman mammal in which recombinant DNA technology has been used to substitute some or all of the amino acids not required for AMH receptor binding with amino acids from corresponding regions of a human immunoglobulin light or heavy chain. A chimeric recombinant antibody homolog is an antibody homolog derived initially from a nonhuman mammal, in which recombinant DNA technology has been used to replace all or part of the hinge and constant regions of the light chain, the heavy chain or both, with corresponding regions from an immunoglobulin light chain or heavy chain of a mammal of a different species, preferably human.

The DNA sequences encoding the AMH receptor may be used as diagnostic tools to determine the extent and rate of the expression of the AMH receptor in cells of a patient. To accomplish this assay, a sample of a patient's cells is treated, via in situ hybridization,

- 11 -

or by other suitable means, and analyzed to determine whether the sample contains mRNA molecules capable of hybridizing with the DNA sequence encoding the AMH receptor.

5 The DNA sequences encoding the AMH receptor may also be used to construct recombinant DNA molecules capable of expressing the AMH polypeptides of this invention in hosts transformed therewith. A DNA
10 sequence encoding an AMH receptor polypeptide of this invention must be operatively linked to an expression control sequence within the recombinant DNA molecule to effect such expression. The term "operatively linked" as used herein refers to positioning in a vector such that transcription and translation of the coding
15 sequence is directed by the control sequence.

 To construct a recombinant DNA molecule capable of directing expression of the AMH receptor polypeptides of this invention, the DNA sequences encoding these polypeptides may be inserted into and
20 expressed using a wide variety of vectors. Furthermore, within each specific expression vector, various sites may be selected for insertion of these DNA sequences. These sites are usually designated by the restriction endonuclease which cuts them. They are
25 well recognized by those of skill in the art. It will be appreciated, however, that an expression vector useful in this invention need not have a restriction endonuclease site for insertion of the chosen DNA fragment. Instead, the vector may be joined to the
30 fragment by alternative means

 The expression vector, and in particular, the site chosen for insertion of a selected DNA fragment and operative linking to an expression control sequence, is determined by a variety of factors. These
35 factors include, e.g., the number of sites susceptible to a particular restriction enzyme, the size of the

- 12 -

polypeptide to be expressed, susceptibility of the desired polypeptide to proteolytic degradation by host cell enzymes, contamination or binding of the polypeptide to be expressed by host cell proteins
5 difficult to remove during purification, expression characteristics, such as the location of start and stop codons relative to the vector sequences, and other factors recognized by those skilled in the art. The choice of vector and an insertion site for a DNA
10 sequence is determined by a balance of these factors and not all selections will be equally effective for a given case.

Useful expression vectors may consist of segments of chromosomal, non-chromosomal and synthetic
15 DNA sequences. Suitable expression vectors for eukaryotic hosts include, for example, vectors comprising sequences from SV40, bovine papilloma virus, adenovirus and cytomegalovirus, and vectors useful specifically in insect cells, such as pVL 941. Useful
20 bacterial expression vectors include known bacterial plasmids, e.g., plasmids from E.coli including colE1, pCR1, pBR322, PMB9 and their derivatives; wider host range plasmids, such as RP4; the numerous derivatives of phage lambda, e.g., NM989 and the lambda gt series;
25 other DNA phages, e.g., M13 and other filamentous single-stranded DNA phages; and commercially available high expression vectors, e.g., the pGEM series and the lambda Zap vectors. Useful mammalian cell expression vectors include, for example, the 2 μ plasmid and
30 derivatives thereof.

Such expression vectors are also characterized by at least one expression control sequence that may be operatively linked to the DNA sequences of this invention inserted in the vector in
35 order to control and to regulate the expression of that cloned DNA sequence. Examples of useful expression

- 13 -

control sequences include the malE system, the OmpA system, the lac system, the trp system, the tac system, the trc system, major operator and promoter regions of phage lambda, the control region of fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase, (e.g., Pho5), the promoters of the yeast mating factors, and promoters derived from polyoma, adenovirus, retrovirus, and simian virus, e.g., the early and late promoters of SV40, eukaryotic cell promoters, such as the metallothionein promoter and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells and their viruses or combinations thereof.

The recombinant DNA molecules of the present invention may also comprise other DNA coding sequences fused to and in frame with the DNA sequences of this invention. For example, such constructs may be characterized by an ATG start codon fused directly to the nucleotides encoding the first amino acid of the mature AMH receptor polypeptide. This construction may produce an f-Met polypeptide. However, it will be understood that the initial methionine may be cleaved during expression in a transformed host or may be subsequently removed. Alternatively, a DNA sequence encoding a bacterial or eukaryotic signal sequence may be fused to the 5' end of a DNA sequence encoding the mature AMH receptor polypeptide of this invention. This would allow the expressed product to be either secreted or targeted to a specific subcellular compartment within the host cell. Most signal sequences are removed by the host cell after performing their targeting function, thus obviating the need for removal after purification of the desired polypeptide. Many signal sequences, as well as the DNA sequences encoding them, are known in the art. The fusion of

- 14 -

such signal sequence DNA to and in frame with the sequence encoding a mature AMH receptor polypeptide of this invention can be achieved by standard molecular biology techniques. Preferably, the signal sequence is
5 selected from the group consisting of nucleotides 62 to 112 of SEQ ID NO: 1; nucleotides 59 to 109 of SEQ ID NO: 2; and nucleotides 61 to 111 of SEQ ID NO: 12.

Alternatively, a DNA sequence encoding an AMH receptor polypeptide of this invention may be expressed
10 as a fusion protein by in-frame ligation to a second DNA sequence encoding a host cell polypeptide. The expression of a fusion protein may afford several advantages, such as increased resistance to host cell degradation, ease of identification based upon the
15 activity or antigenicity of the host cell polypeptide, and ease of purification, based upon the physical or immunological properties of the host cell polypeptide.

This invention also relates to hosts transformed with the recombinant DNA molecules
20 described above. Useful hosts which may be transformed with these recombinant DNA molecules and which may be employed to express the AMH receptor polypeptides of this invention may include well known eukaryotic and prokaryotic hosts, such as strains of E.coli, strains
25 of Pseudomonas; strains of Bacillus; strains of Streptomyces; strains of Saccharomyces; animal cells such as COS cells, CHO cells, BHK cells, R1.1 cells, B-W and L-M cells, African Green Monkey kidney cells (e.g., COS 1, COS 7, BSC1, BSC40 and BMT10); human
30 tissue cells; insect cells (e.g., Spodoptera frugiperda (SF9)); and plant cells in tissue culture. The preferred host for expression of the AMH receptor polypeptides of this invention is CHO cells.

It will be appreciated that not all
35 host/expression vector combinations will function with equal efficiency of expression DNA sequences encoding

- 15 -

the AMH receptor polypeptides of this invention. However, a particular selection of a host-expression vector combination may be made by those of skill in the art after due consideration of the principles set forth
5 herein without departing from the scope of this invention. For example, the selection should be based on a balancing of a number of factors. These factors include, for example, compatibility of the host and vector, toxicity of the polypeptides encoded by the AMH
10 receptor DNA sequences to the host, vector copy number and the ability to control that copy number, the expression of other proteins encoded by the vector, such as antibiotic markers, ease of recovery of the desired polypeptide, expression characteristics of the
15 DNA sequences and the expression control sequences operatively linked to them, biosafety, costs and folding or any other necessary post-expression modifications of the desired polypeptide.

While recombinant DNA techniques are the
20 preferred method of producing the AMH receptor polypeptides of this invention, the AMH receptor DNA sequences of this invention, particularly the DNA sequences encoding only the extracellular domain of the AMH receptor polypeptides, i.e., nucleotides 113 to
25 310 of SEQ ID NO: 1; nucleotides 110 to 490 of SEQ ID NO: 2; and nucleotides 112 to 492 of SEQ ID NO: 12; or sequences which are degenerate to those sequences may be produced by conventional chemical synthesis techniques. Synthetically produced polypeptides of
30 this invention can advantageously be obtained in extremely high yields and be easily purified.

In a preferred embodiment of this invention, AMH receptor polypeptides corresponding only to the extracellular domain (i.e., residues 18 to 83 of SEQ ID
35 NO: 3; residues 18 to 144 of SEQ ID NO: 4 and residues 18 to 144 of SEQ ID NO: 13) are synthesized by solution

- 16 -

phase or solid phase polypeptide synthesis. Proper folding of the polypeptides may be achieved under oxidative conditions which favor disulfide bridge formation, as described by S.Kent, Ann. Rev. Biochem.,
5 57, pp. 957-989 (1988). Polypeptides produced in this way may be purified by separation techniques widely known in the art, preferably utilizing reverse phase HPLC.

In another embodiment of this invention,
10 fusion polypeptides, and DNA sequences coding for them are provided. These fusions have an amino-terminal region characterized by the amino acid sequence of the extracellular domain of the AMH receptor polypeptides of this invention and a carboxy terminal region
15 comprising a domain of a protein or polypeptide other than an AMH receptor polypeptide. Such domains include, for example, the Fc region of an immunoglobulin.

In a preferred embodiment of this invention,
20 the extracellular domain of the AMH receptor polypeptides of this invention are fused to at least a portion of the Fc region of an immunoglobulin. In these fusions, the AMH binding polypeptides form the amino-terminal portion of the fusions, the Fc region
25 forms the carboxy terminal portion of the fusions. The Fc region is preferably limited to the hinge region and the C_H2 and C_H3 domains. The fusion proteins, referred to as an AMH receptor/IgG may be purified from conditioned medium on a Protein A Sepharose column.

30 The AMH receptor polypeptides are useful in radioreceptor assays to measure all bindable forms of AMH. Screening assays of this kind are conventional in the art and any such screening procedure may be employed, whereby the test sample is contacted with the
35 AMH receptors of this invention and the extent of

- 17 -

binding and biological activity of the bound molecule are determined.

The AMH receptor polypeptides of this invention are useful for purifying molecules that bind
5 to an AMH receptor polypeptide, i.e, the AMH ligand. Such purification comprises contacting a sample containing the AMH ligand to be purified with the AMH receptor polypeptide immobilized on a support under
10 conditions in which the AMH ligand is selectively adsorbed onto the immobilized receptor, washing the immobilized support to remove non-adsorbed material and separating the AMH ligand from the immobilized AMH receptor polypeptide to which it is adsorbed.

The AMH receptor polypeptides of this
15 invention may also be used to induce the formation of anti-AMH-receptor antibodies, which are identified by routine screening. Such antibodies may either be polyclonal or monoclonal antibodies, or antigen binding fragments of such antibodies (such as, for example,
20 F(ab) or (Fab)₂ fragments). Of particular significance to the invention are antibodies (and antigen-binding fragments of those antibodies) that bind to the extracellular domain of the AMH receptor polypeptide. The most preferred anti-AMH-receptor antibodies (and
25 antigen-binding fragments thereof) are those capable of binding the receptor in its native conformation on the surface of cells.

Polyclonal antibodies to the AMH receptor polypeptide generally are raised in animals by multiple
30 subcutaneous (sc) or intraperitoneal (ip) injections of the AMH receptor polypeptide and an adjuvant. It may be useful to conjugate the AMH receptor polypeptide (including fragments containing the target amino acid sequence) to a protein that is immunogenic in the
35 species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or

- 18 -

soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine
5 residues), glutaraldehyde, succinic anhydride, or $R^1N=C=NR$, where R and R^1 are different alkyl groups.

The route and schedule of the host animal or cultured antibody-producing cells therefrom are generally in keeping with established and conventional
10 techniques for antibody stimulation and production. While mice are frequently employed as the test model, it is contemplated that any mammalian subject including human subjects or antibody-producing cells obtained therefrom can be manipulated according to the processes
15 of this invention to serve as the basis for production of mammalian, including human, hybrid cell lines.

Animals are typically immunized against the immunogenic conjugates or derivatives by combining 1 mg or 1 μ g of conjugate (for rabbits or mice,
20 respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with 1/5 to 1/10 the original amount of conjugate in Freund's complete adjuvant (for other
25 suitable adjuvant) by subcutaneous injection at multiple sites. Seven to 14 days later animals are bled and the serum is assayed for anti-AMH receptor polypeptide titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the
30 conjugate of the same AMH receptor polypeptide, but conjugated to a different protein and/or through a different cross-linking agent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are used to
35 enhance the immune response.

- 19 -

Monoclonal antibodies are prepared by recovering immune cells, typically spleen cells or lymphocytes from lymph node tissue, from immunized animals and immortalizing the cells in a conventional fashion, e.g., by fusion with myeloma cells or Epstein-Barr virus transformation and screening for clones expressing the desired antibody. The hybridoma technique described originally by Kohler and Milstein, Eur. J. Immunol., 6, p. 511 (1976) and also described by Hammerling et al., Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981) has been widely applied to produce hybrid cell lines that secrete high levels of monoclonal antibodies against many specific antigens. It is possible to fuse cells of one species with another. However, it is preferable that the source of the antibody-producing cells and the myeloma be from the same species.

The hybrid cell lines can be maintained in culture in vitro in cell culture media. The cell lines of this invention can be selected and/or maintained in hypoxanthine-aminopterin thymidine (HAT) medium. In fact, once the hybridoma cell line is established, it can be maintained on a variety of nutritionally adequate media. Moreover, the hybrid cell lines can be stored and preserved in any number of conventional ways, including freezing and storage under liquid nitrogen. Frozen cell lines can be revived and cultured indefinitely with resumed synthesis and secretion of monoclonal antibody.

Monoclonal antibodies may be raised against the extracellular domain of the AMH receptor by immunizing an animal according the above-described procedures with an AMH receptor/IgG fusion protein. Preferably, monoclonal antibodies directed against the extracellular domain will recognize the AMH receptor in its native conformation on the surface of cells and can

- 20 -

be distinguished from those directed against the IgG portion of the fusion protein by standard methods.

Anti-AMH receptor monoclonal antibodies to the extracellular domain of the AMH receptor polypeptide are preferably produced according to the following regime. Animals are first immunized with CHO cells expressing high levels of AMH receptor polypeptides. Prior to fusion of spleen cells with myeloma cells, the animals are boosted with the AMH receptor/IgG fusion protein. Monoclonal antibodies directed against the AMH receptor are identified by standard methods.

The secreted antibody is recovered from tissue culture supernatant by conventional methods such as precipitation, ion exchange chromatography, affinity chromatography, or the like. The antibodies described herein may also be recovered from hybridoma cell cultures by conventional methods for purification of IgG or IgM. The purified antibodies are sterile filtered, and optionally are conjugated with a detectable marker such as an enzyme or spin label for use in diagnostic assays of the AMH-receptor in test samples.

While routinely mouse monoclonal antibodies are used, the invention is not so limited; in fact, human antibodies may be used and may prove to be preferable. Such antibodies can be obtained by using human hybridomas (Cote et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985)). In fact, according to the invention, techniques developed for the production of chimeric antibodies (Morrison et al., PNAS, 81, p. 6851 (1984); Neuberger et al., Nature, 312, p. 604 (1984); Takeda et al., Nature, 314, p. 452 (1985); Shaw et al., J. Nat. Canc. Inst., 80, pp. 1553-1559 (1988); and Oi et al., BioTechniques, 4, p. 214 (1986)) by splicing the genes from a mouse

- 21 -

antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity (such as ability to activate human complement and mediate ADCC) can be used; such antibodies are within the scope of this invention. Also included within the scope of this invention are humanized monoclonal antibodies generated by replacing the complimentary determining regions (CDRs) of a human antibody with the CDRs from an anti-AMH receptor monoclonal antibody.

Techniques for creating recombinant DNA versions of the antigen-binding regions of antibody molecules (known as Fab fragments) which by pass the generation of monoclonal antibodies, are also encompassed within the scope of this invention. This may be accomplished by extracting antibody specific messenger RNA molecules from immune system cells taken from an immunized animal, transcribing these into complementary DNA (cDNA), and cloning the cDNA into a bacterial expression system. One example of such a technique suitable for the practice of this invention incorporates a bacteriophage lambda vector system that contains a leader sequence causing the expressed Fab protein to migrate to the periplasmic space (between the bacterial cell membrane and the cell wall) or to be secreted. One can rapidly generate and screen great numbers of functional Fab fragments for those that bind the antigen. Such AMH-receptor-binding molecules (Fab fragments with specificity for the AMH receptor polypeptide) are specifically encompassed within the term "antibody" as it is defined, discussed, and claimed herein.

The anti-AMH receptor antibodies of the present invention may also be used for diagnostic purposes, such as to measure the expression and function of a patient's AMH receptors. The anti-

- 22 -

receptor antibodies also can be used in imaging to identify and characterize tumors or other tissues, or to define the presence and site of receptor expressing cells.

5 For diagnostic purposes, the receptors and anti-receptor antibodies can be used in accordance with immunoassay technology. Examples of immunoassays are provided by Wide, Radioimmune Assay Method, Kirkham and Huner, Eds., E & S Livingstone, Edinburgh, pp. 199-206
10 (1970).

Thus, in one embodiment, AMH receptor polypeptides can be detectably labeled and incubated with a test sample containing AMH molecules, such as biological fluids and the amount of receptor molecule
15 bound to the sample is ascertained. In a second embodiment, antibody to the AMH receptor polypeptides can be used to create a sandwich type immunoassay. In one such assay, a sample suspected of containing AMH can be incubated in the presence of an immobilized
20 anti-AMH antibody. Solubilized, detectably labeled AMH receptor polypeptides are added to the reaction mixture and the amount of AMH is determined by measuring the amount of bound receptor.

As will be appreciated by those of skill in
25 the art, various alternative assays can also be devised. The assay may be merely diagnostic for the presence of AMH or it may be made quantitative by comparing the measure of labeled molecule with that obtained for a standard sample containing known
30 quantities of AMH.

In another diagnostic test suitable for the AMH receptor polypeptides of this invention involves a single incubation step as the antibody (or receptor) bound to the solid support and labeled receptor (or
35 antibody) are both added to the sample being tested at the same time. After the incubation is completed, the

- 23 -

solid support is washed to remove the residue of fluid sample and uncomplexed labeled antibody. The presence of labeled molecules associated with the solid support is then determined as it would be in a conventional sandwich assay.

Antibodies directed against cell surface antigens such as the AMH receptor also have the capacity to specifically target medical therapies against cancers and tumors in tissues expressing the AMH receptor. The anti-AMH antibody may be effective by itself through antibody dependent and complement dependent cytotoxicity mediated by the Fc domain. Such antibodies can be made more effective as cancer therapeutics by using them as delivery vehicles for drugs, toxins and radionuclides.

One example of an anti-AMH antibody therapy is to conjugate the toxic A chain of ricin or a modified full length form of ricin (which can no longer bind cells) to an antibody directed against the AMH receptor polypeptide expressed on the surface of malignant cells. Such an approach has proved successful with blocked ricin conjugated to a monoclonal antibody against the CD19 antigen expressed on 95% of neoplastic (and normal cells) (Grossbard et al., Blood, 79, p.576 (1992)). As will be appreciated by those of skill in the art, other toxins may be equally useful. This approach should prove even more successful using an anti-AMH receptor antibody because the AMH receptor is only expressed in a very limited number of tissues, i.e., the adult gonads.

Another approach to such medical therapies is to use radioisotope labeled anti-AMH antibodies. Such radiolabeled anti-AMH antibodies will preferentially target radioactivity to tumor sites in cells expressing the AMH receptor, sparing normal tissues. Depending on the radioisotope employed, the radiation emitted from a

- 24 -

radiolabeled antibody bound to a tumor cell may also kill nearby malignant cells that do not express the AMH receptor. A variety of radionuclides may be used. Isotopes that emit β particles (e.g., ^{131}I) have been
5 successful when employed with monoclonal antibodies against CD20 present on B-cell lymphomas (Kaminski et al., N. Engl. J. Med., 329, p. 459 (1993) and Press et al., N. Engl. J. Med., 329, p. 1219 (1993). Radio-
nuclides emitting β particles generate radioactive
10 emissions that are tumoricidal over distances spanning several cell diameters, permitting the eradication of antigen negative cells and diminishing the consequences of inhomogeneous deposition of antibody in tumors.

Radionuclides emitting α particles may also
15 be employed. The low dose rate irradiation generated by radionuclide labeled anti-AMH antibodies may be more therapeutically effective than the instantaneous irradiation delivered externally in conventional radiation therapy. Low dose rate irradiation can
20 induce apoptosis (programmed cell death) in certain cell lines (Macklis et al., Radiat. Res., 130, p. 220 (1992) and Maklis et al., Radiopharm., 5, p. 339 (1992).

The following procedures for isolating AMH
25 receptor DNA sequences and polypeptides according to this invention are set forth for the purposes of illustration only and are not to be construed as limiting the scope of the invention in any manner. Other methods for isolating or preparing the receptor
30 polypeptides of this invention will be apparent to those of skill in the art.

Isolation and Cloning of the AMH Receptor

To isolate and clone the DNA sequences of this invention, we adopted a selection strategy based
35 upon the TGF- β receptor family. Accordingly, we used

- 25 -

64-fold degenerate probe encoding the sequence Tyr Met Ala Pro Glu Val [SEQ ID NO: 5], from a highly conserved region within the serine-threonine kinase domain of the TGF- β type II receptor family to probe a size-selected
5 cDNA library prepared from fetal ovaries. This tissue was chosen for the cDNA library because it responds to AMH by a reduction of aromatase activity. B.Vigier et al., PNAS, 86, pp. 3684-3688 (1989).

Polyadenylated RNA was prepared from 300
10 ovaries obtained from 22-day-old New Zealand rabbit fetuses. J.Chirgwin et al., Biochemistry, 18, pp. 5294-5299 (1979). Double-stranded cDNA was synthesized using M-MLV reverse transcriptase (Superscript Plasmid System, Gibco-BRL, Gaithersburg, Maryland). After
15 addition of non-palindromic BstXI linkers (Librarian kit, Invitrogen, San Diego, California), the cDNA was size-fractionated on a 1% agarose gel and the fraction containing cDNA above 1.6 kb was ligated into the BstXI site of the plasmid vector pCDM8. The ligated DNA was
20 electroporated into E.coli strain MC1061/P3 (B.Seed and A.Aruffo, PNAS, 84, pp. 3365-3369 (1987) and yielded a library of 2.5×10^6 independent clones. The library was subsequently amplified according to standard techniques. From the amplified library, 2×10^6 clones
25 were screened on Gene-screen filters (New England Nuclear, Massachusetts) using the ^{32}P -labelled 64-fold degenerate antisense oligonucleotide probe
5' AC(C/T)TC(A/G/C/T)GG(A/G/C/T)GCCAT(A/G)TA 3'
[SEQ ID NO: 6] that encodes the Tyr Met Ala Pro Glu Val
30 [SEQ ID NO: 5] peptide. R.Cate et al., Cell, 45, p. 685 (1986). The final wash was at 50°C with 3.2M tetramethylammonium chloride/1% SDS.

Seven hundred positive clones were detected by autoradiography and plated in 96 well plates
35 (Costar, Cambridge, Massachusetts). A second round of screening was performed on nitrocellulose filters which

- 26 -

were inoculated with medium from the 96 well plates using a manifold prong. Positive clones were colony purified, DNA was prepared and subjected to dideoxynucleotide sequencing. (F. Sanger et al., PNAS, 5 74, 5463-5467 (1977)). The protein sequences translated from the nucleotide sequences of positive clones was compared to the Genbank/EMBL data bases using the TFASTA program. W.Pearson et al., PNAS, 85, pp. 2444-2448 (1988).

10 Among the positive clones, we identified rabbit cDNAs coding for receptors for several members of the TGF- β family, including the activin receptor type II (ActR-II) (L.Mathews and W.Vale, Cell, 65, p. 973 (1993)), the TGF- β receptor type II (TGF β R-II) 15 (H.Lin et al. Cell, 68, p. 775 (1992)), and the type I receptor identified by R.Ebner et al., Science, 260, pp. 1344-1348 (1993) and W.He et al., Developmental Dynamics, 196, pp. 133-142 (1993). One clone, designated 7F2, clearly belonged to the TGF- β family of 20 receptors but differed from the activin and TGF- β type II receptors and from the type I receptor reported by He et al. Two additional clones related to 7F2 were identified among the 700 positive clones. These clones, designated 2B10 and 3D6, were completely 25 sequenced.

Proteins encoded by clones 7F2, 2B10 and 3D6 are schematically shown in Figure 1A. The longer clone, 3D6, contains a 183 base pair insertion near its 5' end. We believe that this insertion represents an 30 extra exon because it stays in frame throughout (Figures 1B and 1C) and because 3 of its 4 cysteines are aligned with those of either the TGF- β or the activin receptor (Figure 1D). The presence of the extra exon in 3D6 suggests that clone 2B10 is generated 35 by alternate splicing. Clone 3D6 has a shorter 3' untranslated region than 2B10 and contains two introns,

- 27 -

A (159 bp) and B (120 bp). The sequence of these clones in the consensus region VIII (Figure 1D) is Tyr Met Ala Pro Glu Leu [SEQ ID NO: 7] instead of the Tyr Met Ala Pro Glu Val [SEQ ID NO: 5] from which we
5 designed the probes used to screen the library.

To obtain a functional full-length clone, we constructed a hybrid clone, designated H1, by joining the extracellular region of 3D6 to the transmembrane kinase region of 2B10 (Figure 1A) at the BspMI
10 restriction site. The homology of H1 with other TGF- β family receptors was determined according to Kanehisa M. Kanehisa, Nucleic Acids Res., 14, pp. 203-213 (1984) using standard parameters. The intracellular region of the H1 protein exhibits 30.9% homology with human
15 TGFBR-II and 31.9% homology with ActR-II.

Plasmid pB210 is exemplified by a culture deposited in the American Type Culture Collection, Rockville, Maryland on December 16, 1993 and assigned accession number ATCC 69520. Plasmid pH1 is
20 exemplified by a culture deposited in the American Type Culture Collection on December 16, 1993 and assigned accession number ATCC 69521..

Expression Profile of the AMH Receptor

Because AMH exerts its effects solely on the
25 reproductive tract, one would expect its receptor to be expressed only in reproductive organs.

Northern Blot Analysis

A Northern blot analysis of various rabbit tissues at different developmental stages using the
30 following procedures is shown in Figures 2A and 2B.

We obtained various tissues from postnatal male and female rabbits and 22-day-old fetal rabbits. Regression of the male fetal Mullerian duct begins at 20 days post coitum and is significantly advanced at 22
35 days. Puberty in New Zealand rabbits occurs at

- 28 -

approximately 12 weeks. We prepared Sertoli cells from seminiferous tubules isolated from testicular tissue from a 7-week-old rabbit. The Sertoli cells were purified and cultured as described in B.Vigier et al.,
5 Mol. Cell. Endocrinol., 43, pp. 141-150 (1985) except that the Percoll gradient was omitted. Histological examination demonstrated that no germ cells persisted after 5 days in tissue culture.

RNA was isolated as described in J.Chirgwin
10 et al., Biochemistry, 18, pp. 5294-5299 (1979). 10 µg samples were placed in each lane in Figure 2A (except for heart which had 7.65 µg). 20 µg samples were placed in each lane in Figure 2B (except for 4-week-old ovaries which had 18 µg). All RNA samples were
15 electrophoresed on 1% agarose/1% formaldehyde gels, blotted onto Hybond N membranes (Amersham, UK), hybridized as described in R.Cate et al., Cell, 45, pp. 685-698 (1986) with the 1963 bp insert of clone 7F2 removed by digestion with XhoI, and labeled with ³²P by
20 random priming (Megaprime labeling kit, Amersham). The blots were exposed 3 days at -80°C with amplifying screens to Kodak XAR film. Size markers were taken from the 0.24-9.5 kb RNA ladder (Gibco-BRL). After hybridization, the blots were stripped and rehybridized
25 with the 1.1 kb PstI fragment of mouse β-actin cDNA. S.Alonso et al., J. Mol. Evol., 23, pp. 11-22 (1986).

In Figure 2A, a 2,350 b band is observed only in the ovary, the testis and the female fetal reproductive tract; a fainter signal is seen in the
30 male fetal reproductive tract whose Mullerian duct has already significantly regressed. This is the expected expression profile of the AMH receptor. The ontogeny of expression in reproductive tissues is shown in Figure 2B. A strong message is observed in the
35 developing and adult ovary but only faintly during pregnancy. In the testis, a comparable message, not

- 29 -

affected by tissue culture-induced loss of germ cells in seminiferous tubules, disappears at adulthood, indicating that it is expressed in immature Sertoli cells.

5 In Situ Hybridization

To evaluate the specific cells which express the AMH receptor in the reproductive tissues analyzed above, we performed in situ hybridization. The 1963 bp insert of 7F2 was removed by XhoI and cloned at the same site in Bluescript KSII+ plasmid vector (Stratagene, La Jolla, California). ³⁵S-UTP sense and anti-sense RNA probes were produced from T7 and T3 promoters and diluted at 10⁵ cpm μl⁻¹ in hybridization buffer and the in situ hybridization was performed as described in N.Josso et al., Early Hum. Dev., 33, pp. 91-11 (1993). Slides were exposed 10 days at 4°C. After development, the slides were viewed under dark field illumination x 250.

In situ hybridization (Figure 2C) showed that the message was expressed in the mesenchyme surrounding the Mullerian duct and in granulosa cells of ovarian follicles. In the testis, the message was restricted to seminiferous tubules.

Expression of the Two Receptor Isoforms

25 We performed reverse-transcriptase polymerase chain reaction (RT-PCR) to determine the relative levels of the H1 and 2B10 mRNAs in AMH target tissues according to the following procedure.

RT-PCR was run with 200 units of M-MLV reverse transcriptase in 40 μl of the buffer supplied by the manufacturer (Gibco-BRL) containing 1.25 mM dNTP, with 5 μg total RNA and 200 pmol random hexanucleotide primer, for 45 minutes at 42°C. A 4 μl aliquot of this reaction product was directly used for PCR amplification, as described in B.Knebelmann et al. PNAS, 88, pp. 3767-3771 (1991) except that magnesium

- 30 -

concentration of the buffer was lowered to 1.34 mM. Twenty pmol of each of the two oligonucleotides, 5' GCAGGATGCT GGGCACTCTG 3' [SEQ ID NO: 8] and 5' GTCAGCACCA CAGGAGCAGG 3' [SEQ ID NO: 9] flanking the extra exon were used (see Figure 4A), in a 100 μ l reaction. Amplification was carried out for 30 cycles of denaturation at 95°C for 45 seconds, annealing at 60°C for 45 seconds, and elongation at 72°C for 75 seconds. For control amplification, 50 ng of DNA of clones or 0.5 μ g rabbit genomic DNA were used. 15 μ l of PCR product were electrophoresed on 12% acrylamide:bis 39:1 gels and stained by ethidium bromide.

As shown in Figure 4, both H1 and 2B10 are expressed in AMH target tissues with a slight prevalence of the H1 isoform. All control tissues are negative except male fetal liver, which expresses low amounts of the shorter 2B10 isoform. The identity of the PCR bands was checked by blot hybridization using labeled internal oligonucleotides indicated by asterisks in Figure 4A. The 5' oligonucleotide, 5' CGCAGGAAGC AGTGCCCAA 3' [SEQ ID NO: 10] hybridizes with both the 164 and the 347 bp bands. The other oligonucleotide, 5' ACACACAGGT CCTCCTGTTT 3' [SEQ ID NO: 11] hybridizes only with the 347 bp band, specific to the extra exon. The band corresponding to contaminant genomic DNA begins to emerge in cDNA samples which do not contain significant amounts of receptor cDNA(s).

30 Binding of Iodinated AMH to Clone H1 Expressed in COS cells

To confirm that clones H1 and 2B10 encode the AMH receptor, we introduced these clones into COS cells to determine if the transfected cells bind to AMH. AMH must be cleaved near the C-terminus for bioactivity and

- 31 -

the N-terminal fragment must remain associated with the C-terminal fragment for full activity. C.Wilson et al., Mol. Endocrinol., 7, pp. 247-257 (1993).

Therefore, binding studies were carried out with AMH
5 cleaved by plasmin, which generates a non-covalent complex of the N and C-terminal fragments. This complex is extremely sensitive to iodination and loses bioactivity at specific activities above 4 $\mu\text{Ci } \mu\text{g}^{-1}$.

Full-length and plasmin-cleaved AMH
10 preparations (C.Wilson et al., Mol. Endocrinol., 7, pp. 247-257 (1993)) were iodinated by chloramine T Oxidation as described in C.Frolik et al., J. Biol. Chem., 259, pp. 10995-11000 (1984). The resulting specific activity was approximately 15-25 $\mu\text{Ci } \mu\text{g}^{-1}$ and
15 1-4 $\mu\text{Ci } \mu\text{g}^{-1}$ for full-length and plasmin-cleaved AMH, respectively. The bioactivity of the labeled preparations, checked by the anti-aromatase assay N.di Clemente et al., Development, 114, pp. 721-727 (1992) after sham labeling with cold iodine, was at least 70%
20 of the unlabeled control preparation. COS-M6 cells (3×10^5) were plated on either poly-D-lysine-coated one-chambered Labtek microscope slides or 6-well plates (Costar, Cambridge, Massachusetts). Twenty four hours later, cells were transfected by the DEAE/chloroquine
25 procedure as described in A.Aruffo and B.Seed, PNAS, 84, pp. 8573-8577 (1987) except that all reagents were added to the culture medium at the same time. The cells were cultured 3 days in Eagle's minimum essential medium containing 10% female fetal serum and exposed 4
30 hours at 4°C to iodinated AMH in RPMI medium (Eurobio, Les Ulis, France) containing 0.5% bovine serum albumin. Cells on Labtek slides were exposed to a 1 nM concentration of either full-length or plasmin-cleaved AMH and prepared for autoradiography as described in
35 L.Mathews and W.Vale, Cell, 65, p. 973 (1993). After 10 days, the slides were developed, dehydrated, stained

- 32 -

with toluidine blue and examined under dark field illumination. Transfection efficiency was approximately 30%, as determined by transfection with β -galactosidase DNA and staining with 5-bromo-4-chloro-3-indolyl- β -galactopyranoside.

Results of binding studies with labeled AMH preparations are shown in Figure 3. COS cells transfected with clone H1 bind plasmin-cleaved but not full-length AMH; cells transfected with the short receptor isoform 2B10 or with β -galactosidase DNA do not bind plasmin-cleaved AMH. These results indicate that H1 encodes a receptor that is competent for AMH binding, while the truncated form encoded by 2B10 is not.

To assure that these results were statistically significant, transfected cells (prepared as described above) cultured in 6-well dishes were exposed to 0.5, 1, or 2 nM iodinated plasmin-cleaved AMH, with or without a 100-fold excess of the cold ligand as described in L.Mathews and W.Vale, Cell, 65, p. 973 (1993) and displaceable binding was measured. The results are recorded in Table 1.

Table 1

| AMH conc. (nM) | sp act $\mu\text{Ci}/\mu\text{g}$ | displaceable counts per min | | |
|-------------------|--------------------------------------|-----------------------------|------|-------|
| | | H1 | 2B10 | 3F11* |
| 0.5 | 2.6 | 1898 | | 1343 |
| 0.5 | 4.1 | 1846 | 133 | 570 |
| 1.0 | 1.0 | 306 | | 121 |
| 1.0 | 1.0 | 228 | | -56 |
| 1.0 | 1.0 | 516 | | 17 |
| 1.0 | 2.6 | 2073 | 1527 | 970 |
| 1.0 | 4.1 | 2992 | 1692 | 2457 |
| 2.0 | 2.6 | 3496 | 1681 | 2480 |

* 3F11 is full length cDNA of rabbit TGF β R-II

Displaceable counts represent the difference between mean counts bound to cells exposed only to

- 33 -

labeled plasmin-cleaved AMH and that of counts bound to cells exposed to both labeled and a 100-fold excess of cold ligand, each determined in triplicate. The number of counts corresponding to 1 nM of labeled ligand
5 varied between 150,000 and 1,260,000 cpm, according to the specific activity. In the four instances where the three clones were tested in the same experiment, Student's paired t test analysis shows no significant difference between displaceable binding to clones 2B10
10 or 3F11 ($p=0.337$). In contrast, displaceable binding to clone H1 is significantly higher than that to either 2B10 ($p=0.019$) or 3F11 ($p=0.008$) transfected cells. When H1 was compared to either 2B10 or 3F11 ($n=12$), the difference was even more striking ($p< 0.001$).

15 Isolation of the Human AMH Receptor cDNA

To isolate the cDNA for the human AMH receptor, we screened a human testis cDNA library with a DNA probe derived from the rabbit AMH receptor cDNA clone H1. The human testis library was made from RNA
20 isolated from human testis obtained from a 6 month old patient with androgen insensitivity. Chirgwin, Biochemistry, 18, pp. 5294-5299 (1979). Double stranded cDNA was synthesized from polyadenylated testis RNA using the Time Saver kit from Pharmacia.
25 After addition of EcoR1/Not1 linkers, the cDNA was size selected and ligated into the EcoR1 site of λ gt11. Aliquots of the ligation were packaged into phage particles using Gigapack II (Stratagene). The packaged DNA was used to infect E. coli 1090 cells. Plating of
30 the library yielded 2.6×10^6 independent plaques and was subsequently amplified. The titre of the phage library was 8.5×10^{10} pfu/ml.

We screened the library with a random primed probe derived from the rabbit AMH receptor cDNA clone
35 H1, using the plaque hybridization screening technique

- 34 -

of Benton and Davis (Science, 196, p. 180 (1977)). 2 X
10⁶ plaques were screened on Hybond N (Amersham) filters
with the ³²P-labeled probe. The 2494 bp probe was
derived from clone H1 by digesting plasmid H1 with
5 XhoI, purified on an agarose gel, and ³²P-labeled using
the random priming method (Megaprime labeling kit,
Amersham). Conditions were standard, and the final
washing conditions were 2 X SSC, 0.1% SDS at 55°. We
detected positive hybridizing clones by
10 autoradiography. These plaques were isolated and
rescreened at lower density, until completely pure.

DNA was purified from one of the positive
clones, designated λ-hAMHR-3. The insert was removed
with EcoRI and cloned into the EcoRI site of plasmid
15 Bluescript KS II (+). The resulting plasmid was
designated KS-hAMHR3-2. The insert was completely
sequenced by the method of Sanger et al. and is shown
in SEQ ID NO: 12. A comparison of the predicted amino
acid sequence with that of the rabbit AMH receptor is
20 shown in Figure 5 [SEQ ID NO: 4 AND SEQ ID NO 13]. The
two proteins share 82% similarity, indicating that
clone 3-2 encodes the human AMH receptor.

Plasmid hAMHR3-2 is exemplified by a culture
deposited in the American Type Culture Collection,
25 Rockville, Maryland on December 13, 1994 and assigned
accession number ATCC _____.

Genetic Evidence that Clone 3-2 Encodes
the Human AMH Receptor

In order to prove that clone 3-2 encodes the
30 human AMH receptor, we analyzed the gene that encodes
the 3-2 cDNA in normal humans and in humans who suffer
from possible mutations in the AMH receptor. These
males are externally virilized but retain a uterus and
fallopian tubes, a condition termed Persistent
35 Mullerian duct syndrome (PMDS). It has been shown that

- 35 -

in some of these patients, the gene for AMH contains mutations, rendering the AMH that is produced nonfunctional. Imbeaud et al., Hum. Mol. Genet., 3, pp. 125-131 (1994). This accounts for the persistence of the Mullerian duct in these individuals, since no functional AMH is present to cause the regression of the Mullerian duct during fetal development. However, some individuals that suffer from PMDS have completely normal AMH, indicating that their AMH receptor may be nonfunctional due to mutations. Thus we wanted to analyze the gene that encodes the 3-2 cDNA in such individuals to see if they did indeed contain a mutation.

A portion of the gene was analyzed in a 2.6 year old patient (Patient T.A.) with AMH positive (i.e. functional AMH could be detected in a testicular biopsy sample) PMDS. Single strand conformational polymorphism (SSCP) analysis [Orita et al., PNAS, 86, pp. 2766-2770 (1989)] was performed on PCR products generated from DNA isolated from lymphocytes obtained from patient T.A. and from a normal human male, using primers designed from the 3-2 cDNA clone sequence. This analysis allows the detection of single base changes. The SSCP analysis detected a polymorphism in a portion of the gene that encodes the extracellular domain of the receptor. Two PCR primers (1s [SEQ ID NO: 15] and 2a [SEQ ID NO: 16]) were then used to amplify the portion of the gene which contained this polymorphism. The PCR product was cloned into the pGEM-T cloning vector using the AT cloning method of Promega and sequenced. The sequence is shown in Figure 6 [nucleotides 401-800 of SEQ ID NO: 14]. The sequence covers an exon from the extracellular domain and an adjacent intron. At the junction of the exon and intron, one finds the dinucleotide sequence AT, instead of the GT dinucleotide sequence found at the 5'

- 36 -

end all introns, indicating that the receptor gene in patient T.A. contains a splicing mutation.

To confirm that this change is indeed a mutation, we cloned a portion of the gene from a human
5 genomic λ EMBL4 library. This library was made with genomic DNA isolated from normal human muscle, thus any 3-2 receptor gene isolated from this library should have a normal (i.e. wild type) sequence. The gene was cloned from the λ EMBL4 library using standard
10 conditions, and using the insert from clone 3-2 as a probe. A clone (51bb) containing the 5' portion of the gene was isolated, DNA was purified, and a 8 kb EcoR1 fragment was excised and subcloned into the vector Bluescript KS II (+) to generate clone 3-35. A partial
15 nucleotide sequence derived from clone 3-35 is shown in Figure 7 [SEQ ID NO: 14]. It covers the first two exons and a portion of the third exon, all of which are within the extracellular domain of the receptor. The sequence also contains two introns, shown in lower
20 case. Both introns begin with the dinucleotide GT and end with the dinucleotide AG. It is the GT dinucleotide in the second intron shown in Figure 7 [SEQ ID NO: 14], which has been mutated in the gene of patient T.A.

25 Furthermore, both receptor genes of patient T.A. contain this mutation, which is consistent with the fact that PMDS is an autosomal recessive genetic disease (i.e. the function of both genes must be eliminated in order to see a phenotype). The G>A
30 mutation destroys an Hph 1 site; thus one can assess the presence of the mutation by digesting the PCR fragment (generated with primers 1s [SEQ ID NO: 15] and 2a [SEQ ID NO: 16]) with Hph 1. The PCR fragment made from normal DNA was completely digested with Hph 1,
35 while the PCR fragment made from patient T.A. DNA was resistant to digestion with Hph 1, indicating that both

- 37 -

alleles contain the G>A mutation. This implies that the patient inherited one mutant gene from each parent. Indeed, an Hph 1 digestion of the PCR fragment made from the DNA of the mother and father indicated that
5 only 50% of the fragment could be digested (i.e. both the mother and father have one normal gene and one mutant gene).

In order to demonstrate that the mutant gene causes a problem with splicing, RNAs isolated from a
10 testicular biopsy of patient T.A. and from normal human fetal testis were subjected to RT-PCR using primers 1s [SEQ ID NO: 15] and 3a [SEQ ID NO: 17]. The PCR products were analyzed by agarose gel electrophoresis (Figure 8); they were also cloned into the pGEM-T
15 vector using the AT cloning method of Promega and sequenced. Only one fragment was produced with RNA from the normal samples, while two variant PCR products were produced from patient T.A. RNA (Figure 8). The large fragment was shown by sequencing to contain a
20 portion of the intron (12 bp), reflecting an aberrant mRNA that was generated by use of a cryptic splice donor site downstream of the mutated donor site (Figure 9). The smaller variant PCR fragment was shown by sequencing to be missing the second exon; this product
25 reflects an mRNA that has undergone exon skipping. The smaller mRNA would be expected to produce a non-functional receptor, since it is identical to the alternately spliced receptor (2B10) produced in the rabbit, which we have shown cannot bind AMH. The mRNA
30 that is produced by cryptic splicing would contain a 4 amino acid insertion in the middle of the extracellular domain, and would also be expected to be non-functional.

Therefore, the presence of a splicing mutation
35 in both copies of the 3-2 gene in patient T.A. who

- 38 -

suffers from PMDS, confirms that the 3-2 gene encodes the AMH receptor.

Expression of the AMH receptor in a Tumor Sample

To demonstrate that the AMH receptor can be
5 used to direct a toxin to a tumor cell, it is necessary
to show that tumors express the AMH receptor. We have
used RT-PCR to show that four granulosa cell tumors do
express the AMH receptor. RNA was isolated from the
tumor samples and subjected to RT-PCR using two primers
10 (1s [SEQ ID NO: 15] and 2a [SEQ ID NO: 16]) from the
human AMH receptor cDNA sequence. As shown in
Figure 10, a PCR product of the expected size is
generated from the RNA of these tumor samples.

Therefore, a toxin coupled to an antibody
15 against the AMH receptor can be used to eradicate these
tumors.

- 39 -

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: BIOGEN, INC.
CATE, Richard L.
INSERM, (U.293)
JOSSO, Nathalie
- (ii) TITLE OF INVENTION: ANTI-MULLERIAN HORMONE RECEPTOR
POLYPEPTIDES AND ANTIBODIES THERETO
- (iii) NUMBER OF SEQUENCES: 17
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: c/o FLSH & NEAVE
 - (B) STREET: 1251 Avenue of the Americas
 - (C) CITY: New York
 - (D) STATE: New York
 - (E) COUNTRY: U.S.A.
 - (F) ZIP: 10020
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/166,333
 - (B) FILING DATE: 13-DEC-1993
- (viii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/173,512
 - (B) FILING DATE: 23-DEC-1993
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Haley Jr., James F.
 - (B) REGISTRATION NUMBER: 27,794
 - (C) REFERENCE/DOCKET NUMBER: B174CIP
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (212) 596-9000
 - (B) TELEFAX: (212) 596-9090

- 40 -

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2228 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: misc feature
- (B) LOCATION: 1..2228
- (D) OTHER INFORMATION: /note= "clone 2B10"

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 62..112

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
- (B) LOCATION: 113..1585

(ix) FEATURE:

- (A) NAME/KEY: misc feature
- (B) LOCATION: 113..310
- (D) OTHER INFORMATION: /note= "extracellular domain"

(ix) FEATURE:

- (A) NAME/KEY: misc feature
- (B) LOCATION: 311..388
- (D) OTHER INFORMATION: /note= "transmembrane region"

(ix) FEATURE:

- (A) NAME/KEY: misc feature
- (B) LOCATION: 389..1585
- (D) OTHER INFORMATION: /note= "cytoplasmic domain"

(ix) FEATURE:

- (A) NAME/KEY: misc feature
- (B) LOCATION: 200..205
- (D) OTHER INFORMATION: /note= "BspMI restriction site"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```

AAAGGAGAGC CTGGGCGGCG TGGCTGGTGC TCTCTGCG AGGCTGGCAC CTTGGGGCAG      60
GATGCTGGGC ACTCTGGGGC TTGGGGCACT GCTTCTGCG GCTGTGCAGG GATGCGGAGA      120
CAGTGAAGAG CCAGGCTGTG AGTTCCTTAG CTGTGAACCG AGCCCCGAG CCGGCGGACG      180
CTCTGGCTCC ACTCTCTTCA CCTGCTCTTG TGGTGCAGAC TTCTGCAATG CCAACTACAG      240
CCATCTGGCT CTTCTGGGGG GCGCTGGGAC TCTTGGGCCC CAGGGTCCCC AGGCTGCCCC      300

```

- 41 -

| | |
|---|------|
| AGGTGAGTCC CCGTGGATGG CACTGGGGCT GCTGGGGCTG GTCTGGCTGC TGTGGCTGCT | 360 |
| GCCTGGGGGT ATTGTGTGTG CTCTGCTCCA GCGAAAGGOC TACAGGGTGC AGAGTGGGOC | 420 |
| AGAGGACAGC CCAGACTCAG GCAGGGACTG CAGTGAAGAG CTGGGGGAGC TGGGGGAGCT | 480 |
| GTCCTCTCC CAGGTTCATC GCGAAGGAGG TCAGGGGCA GTGTGGGGCTG GGCAGCTCCA | 540 |
| AGGGGAGCTG GTGGGATCA AGGTCTCTCC CCGGAGGGOC GTGGGGAGT TGGGAGCTGA | 600 |
| GAGAGGCTG TATGAGCTGC CCGGGCTCCA GCACAACAC GTGTGGGAT TTATGGCTGC | 660 |
| TGGGAGGGG GGAAGGGOC CCGGGGCTC TGGGGGGCTG CTGGTACTGC AACTGCACCC | 720 |
| CAGGGGCTC CTGTGGAGT ACGTGAACA GCACAAGT GACGGGGGA GTTGGGAG | 780 |
| GATGGCTCTG TCTTTAGGOC AGGGGCTGGC ATTTCTCAT GAGGAGGCT GCGAGGATG | 840 |
| CCAGTACAAG CCGGTATG CCGAGGAGA TCTGAGGAGC CAGATGTGC TCATGGGGA | 900 |
| AGATGGGTCA TGTGGATG GAGAGGGG CCGGGGCTG GTCTGGGCT GTTGGCTCA | 960 |
| GGGGGCTC TGGGGGCTC GCGAGGGG AGGGGAGG GGCATCATG AGGGGGCAC | 1020 |
| ACAGAGGTAC ATGGGGGAG AGCTCTGGA CAGTCTCTG GACCTACAGG ACTGGGGCAC | 1080 |
| TGGGGGGG GAGGGGAG TCTACTCTT GGGGCTCTC CTGTGGGAGA TCTGAGGG | 1140 |
| CTGGGGGAT TGTGGGCTG AGGGGAGC ACGGGGCTC CAGTGGCT ATTGAGGAGA | 1200 |
| ACTGGGAGC GGGGGACA CCGTGTGCT GTGGGGGCTG GCAGTGAAG AGAGGAGG | 1260 |
| CCGTGATC CCATCTCTT GTGGGCTT TGGGAGAC CCGGGGGC TCAGGGAGCT | 1320 |
| GCCTGAAGAC TGTGGGAG CAGAGGGG AGGGGGCTG AGGGGGAGT GTGTGGGCA | 1380 |
| GGGGGGGT GGGGGGCT ATCTGAGG GGGGAGG TGGGAGG GGGGTGACA | 1440 |
| CAGGAGGA GAGAGTGC CCGGGCTC TGGGGGCT CCGCTCTCC TGGGGGAG | 1500 |
| CCGAGGGG GTGGGCTC ACTGGGTG TCAGGAGG CTTGCTCA GGAAGGGG | 1560 |
| AGCTGGCTGT GCGATCTG AGGTGAAAT AAGGATTTG TGTGTAATCT ACGTGAAC | 1620 |
| GTAACATGG CACTGGTATA CCGTCTGCT CTGGCTCTC ACTGTTTCC CACTGGGCA | 1680 |
| TCGTGAGG AGGAGGCTG AATGTGAGC TATGTGTGTG TGTGTGAGC AGGGCTGAG | 1740 |
| ACGAGGAGC ATTGAGGAG CCGGAGAA CCGGATCTT GCACTCAGA GTCTGGGCA | 1800 |
| GAGGCTCA ACTGGGAG TGTGGGAG CTTGGGCTA AGGCAAGCT CAGGAGAT | 1860 |
| TCTCTCTCT CAGGAGGAA GGGGTACTT GATCTGATA CAGGGGAT AAAAGCTCA | 1920 |

- 42 -

```

CAGAGTCCC TTGCAGCAA AACTGGGGAG GAATTTCGAA ATTTCACCTC AACAAACCTT 1980
TCAACAGGG CTCCCCCTTC TTGTCTCTGG AGGAGAAAGG GAGGTGGGTA AACAGACTCC 2040
CTTAAAAAOC TAGGGAGTCC AAACGAGCA CACACACAGC CCTCTGCTTC TCTGCTTACG 2100
CGCCCCGCTG GCTGCCCCAG GGTATCTCTC TTCAATCAAC CATGTAAGCT TCCCTCTTCC 2160
CTGAGCGCTC TCTGTCTCTC CCTTGGTTT TGACAGATGC CCTGTCCCCA ATAAACCTTA 2220
TCATCTCTG 2228

```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2408 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: misc feature
- (B) LOCATION: 1..2408
- (D) OTHER INFORMATION: /note= "clone H1"

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 59..109

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
- (B) LOCATION: 110..1765

(ix) FEATURE:

- (A) NAME/KEY: misc feature
- (B) LOCATION: 110..490
- (D) OTHER INFORMATION: /note= "extracellular domain"

(ix) FEATURE:

- (A) NAME/KEY: misc feature
- (B) LOCATION: 491..568
- (D) OTHER INFORMATION: /note= "transmembrane region"

(ix) FEATURE:

- (A) NAME/KEY: misc feature
- (B) LOCATION: 569..1765
- (D) OTHER INFORMATION: /note= "cytoplasmic domain"

- 43 -

(ix) FEATURE:

(A) NAME/KEY: misc feature

(B) LOCATION: 108..290

(D) OTHER INFORMATION: /note= "extra exon missing in 2B10"

(ix) FEATURE:

(A) NAME/KEY: misc feature

(B) LOCATION: 380..385

(D) OTHER INFORMATION: /note= "BspM1 restriction site"

(ix) FEATURE:

(A) NAME/KEY: misc feature

(B) LOCATION: 2390..2395

(D) OTHER INFORMATION: /note= "poly A signal sequence"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

GGGAGGCGTG GGGGCGCTGG CTGGGCTCTT CCGCAGGGG CTGCCAAGCT GGGCAGGAT      60
GCTGGGCACT CTGGGCTTT GGGCAGCTT TCTGGGCT GTGCAGGCAC CCGCAACAG      120
GAGCACTGT GTGTCTTTG AGGCGCTGG AGTGGGGGA AGCACAAGA CACTGGGGGA      180
GCTGCTAGAT GCAGGACAG GGGGGGAG GGTATGCG TGCTCTACA GCGCTGCTG      240
CTTGGGATC TGAAGCTAA CCGGAGACA GGCACGGTG GAGATGCAAG GATGCGAGA      300
CAGTACAG CCAGGCTG AGTGGCTAG CTGTAGGAG AGGGGGAG CCGGCGAG      360
CTCTGGCTC ACTCTCTCA CCGCTCTG TGGTCTGAC TTCGCAATG CCACTACAG      420
CCATCTGCT CCGTGGGGG GCGTGGGAC TCTGGGCG CAGGCTGCG AGGCTGCGC      480
AGGTAGTCT CCGTGGGAG CACTGGGCT GCTGGGGTG GTCTGCTG TCGTCTGCT      540
GCTGGGGGT ATTGTGCTG CTCTCTCA GCGAAGGCT TACAGGCTC AGAGTGGGC      600
AGAGCAGAG CCAGCTCAG GCAGGCTG CAGTAGGAG CTGGGGAGC TCGGCGAGT      660
GTCTCTCTC CAGGCTCTC GCGAAGGAG TCAGGGGCA GTGTGGGCT GGCAGCTCA      720
AGGGGAGCT GTGGCATCA AGGTCTTCC CCGAGGGCT GTGGGCGAT TCGAGCTCA      780
GAGAGCTTG TATGAGCTG CCGGCTGCA GCACAACAC GTGTGCGAT TTATGCTG      840
TGGCAGGGG GGAAGGGCT CCGTGGCTC TGGGGGCTG CTGTACTG AACGCAAGC      900
CAAGGGCTC CTGTGCGAT ACGTAGACA GCACAAGT GACTGGGGA GTTGGCTG      960
GATGGCTCT TCTTAGGCT AGGGCTGGC ATTCTCAT GAGGAGGCT GGCAGGATG      1020
CCAGTACAG CCGGTATG CCGAGGAGA TCTAGGAGC CAGATGCTC TCATGGGGA      1080
AGATGGGCT TGTGCTG GAGAGCTGG CCGGCTG GTGCTGCTG GTTGGCTCA      1140

```

- 44 -

| | |
|--|------|
| G0000G1G0C TGGG0000TC GGCAG0000G AGG000CAGG GGCATCATGG AGG000GGCAG | 1200 |
| ACACAGGTAC ATGGG00CAG AGCTCTTGA CAAGTCTCIG GA0CTACAGG ACTGGGGCAG | 1260 |
| TG00CT00GG CGAG00GAG TCTACT0CTT G000CTGCTC CTG1GGGAGA T0CTGAG00G | 1320 |
| CTG000GGAT TTGAGG00TG A0GGCAGAC ACCA00CTTC CAAC1GG0CT ATGAAGCAGA | 1380 |
| ACTGGGCAGC G0000CA0CA 0CTGTGAGCT G1GGG00CTG GCAGTAGAGG AGAGGAGGGG | 1440 |
| 00CTGACATC 0CAT0CT0CT GG1GCTGCTT TG00CAGAC 00GG0GGGGC TCAGGGAGCT | 1500 |
| GCTGGAGAC TGCTGGGAG CAGA000GA AG0GGGGCTG A0GG00GAGT G1GT0CAGA | 1560 |
| G0G0CTGG1G G00CTGG1TC AT0CTCAGA G000CAG00C TG00CAGAGG G0GTC0ACA | 1620 |
| CAG0CA00CA GAAGACTGGC 000CTGCT0C TG000CTG0C 0CTGCTCT0C T000GGCAG | 1680 |
| 00CAGAG00G GGTG0CTG0C ACT0GGG1GT TCAGCAAGG C1TTGCT0CA GGA0000GG | 1740 |
| AGCTG0CTGT G0CAGT1CTG A0G1G1AAAT AAGCAGTTG TG1GTAATCT A0CTGTAAAC | 1800 |
| GTAAACATGG CACTGGTATA 0CTGTCTGCT CTG0CTCT0C ACTGTTT0C CACT000CAA | 1860 |
| TC1GTTAGG CAGGAGCTGG AAATTGAGC TATG1G1G1G TG1G1GAGC AGG0CTGAAG | 1920 |
| A0CAGCA00C ATTGCAGAAG 000CAGAAAC 0CAGCATCTT GCAC1TCAGA GT0CTG00CA | 1980 |
| GA00CTGCTA A0CT00CAGG TGGT00CAGC 0CTT00CTCA AGGCAAACTC 0CAGGAGAAT | 2040 |
| TC1CTCT0CT CAGGACAAA GGGGTTA0CT GATCTGATAA CACAGGGCAT AAAA0CTCA | 2100 |
| CAGAGT000C TTG0CAGCA A0CTGGGGAG GAATTGCAA ATTTTCAC1C AACAA00CT | 2160 |
| T0CA0CAGGG CT00000CTC TT1GT0CTGG AGGAGAAAGG GAGG1GGGTA AACAGACT0C | 2220 |
| CTTAAAA0C TAGGGAGT0C AAAC1GA0CA 0GCACTCAGC 0CTCTG0CTC TCTG0CTAGC | 2280 |
| 0G00GG0CTG G0CTG00CAG G1G1ATTCTC TTCA1TCAAC CATG1GA0CT TGCTCTT00C | 2340 |
| CTGAGGGCTC TC1GTCTGTC 00CT00GTTT TGACAGATGC 0CTGT000CA ATAA0CTTA | 2400 |
| TCACTCTG | 2408 |

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 508 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

- 45 -

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Domain

(B) LOCATION: 18..83

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION: 18..508

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Leu Gly Thr Leu Gly Leu Trp Ala Leu Leu Pro Ala Ala Val Gln
 1 5 10 15

Gly Cys Arg Asp Ser Asp Glu Pro Gly Cys Glu Ser Leu Ser Cys Asp
 20 25 30

Pro Ser Pro Arg Ala Arg Ala Ser Ser Gly Ser Thr Leu Phe Thr Cys
 35 40 45

Ser Cys Gly Ala Asp Phe Cys Asn Ala Asn Tyr Ser His Leu Pro Pro
 50 55 60

Leu Gly Gly Pro Gly Thr Pro Gly Pro Gln Gly Pro Gln Ala Ala Pro
 65 70 75 80

Gly Glu Ser Pro Trp Met Ala Leu Ala Leu Leu Gly Leu Val Leu Leu
 85 90 95

Leu Leu Leu Leu Leu Gly Gly Ile Val Val Ala Leu Leu Gln Arg Lys
 100 105 110

Ala Tyr Arg Val Gln Ser Gly Pro Glu Pro Glu Pro Asp Ser Gly Arg
 115 120 125

Asp Cys Ser Glu Glu Leu Pro Glu Leu Pro Gln Leu Cys Phe Ser Gln
 130 135 140

Val Ile Arg Glu Gly Gly His Ala Ala Val Trp Ala Gly Gln Leu Gln
 145 150 155 160

Gly Glu Leu Val Ala Ile Lys Val Phe Pro Arg Arg Ala Val Ala Gln
 165 170 175

Phe Arg Ala Glu Arg Ala Leu Tyr Glu Leu Pro Gly Leu Gln His Asn
 180 185 190

His Val Val Arg Phe Ile Ala Ala Gly Gln Gly Gly Pro Gly Pro Leu
 195 200 205

Pro Ser Gly Pro Leu Leu Val Leu Glu Leu His Pro Lys Gly Ser Leu
 210 215 220

- 46 -

Cys Gln Tyr Leu Ser Gln His Thr Ser Asp Trp Gly Ser Ser Leu Arg
 225 230 235 240
 Met Ala Leu Ser Leu Ala Gln Gly Leu Ala Phe Leu His Glu Glu Arg
 245 250 255
 Trp Gln Asp Gly Gln Tyr Lys Pro Gly Ile Ala His Arg Asp Leu Ser
 260 265 270
 Ser Gln Asn Val Leu Ile Arg Glu Asp Gly Ser Cys Ala Ile Gly Asp
 275 280 285
 Leu Gly Leu Ala Leu Val Leu Pro Gly Phe Ala Gln Pro Arg Ala Trp
 290 295 300
 Ala Pro Pro Gln Pro Arg Gly Pro Ala Ala Ile Met Glu Ala Gly Thr
 305 310 315 320
 Gln Arg Tyr Met Ala Pro Glu Leu Leu Asp Lys Ser Leu Asp Leu Gln
 325 330 335
 Asp Trp Gly Thr Ala Leu Arg Arg Ala Asp Val Tyr Ser Leu Ala Leu
 340 345 350
 Leu Leu Trp Glu Ile Leu Ser Arg Cys Pro Asp Leu Arg Pro Asp Gly
 355 360 365
 Arg Pro Pro Pro Phe Gln Leu Ala Tyr Glu Ala Glu Leu Gly Ser Ala
 370 375 380
 Pro Thr Thr Cys Glu Leu Trp Ala Leu Ala Val Glu Glu Arg Arg Arg
 385 390 395 400
 Pro Asp Ile Pro Ser Ser Trp Cys Cys Phe Ala Thr Asp Pro Gly Gly
 405 410 415
 Leu Arg Glu Leu Leu Glu Asp Cys Trp Asp Ala Asp Pro Glu Ala Arg
 420 425 430
 Leu Thr Ala Glu Cys Val Gln Gln Arg Leu Val Ala Leu Val His Pro
 435 440 445
 Gln Glu Ala Gln Pro Cys Pro Glu Gly Arg Pro His Ser His Pro Glu
 450 455 460
 Asp Trp Pro Pro Ala Pro Ala Pro Ala Pro Ala Leu Leu Pro Gly Ser
 465 470 475 480
 Pro Gln Pro Gly Ala Cys His Phe Gly Val Gln Gln Gly Leu Cys Ser
 485 490 495
 Arg Asn Pro Gly Ala Ala Cys Ala Ser Ser Asp Val
 500 505

- 47 -

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 569 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Domain
- (B) LOCATION: 18..144

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION: 18..569

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

Met Leu Gly Thr Leu Gly Leu Trp Ala Leu Leu Pro Ala Ala Val Gln
 1             5             10             15

Ala Pro Pro Asn Arg Arg Thr Cys Val Phe Phe Glu Ala Pro Gly Val
      20             25             30

Arg Gly Ser Thr Lys Thr Leu Gly Glu Leu Leu Asp Ala Gly Pro Gly
      35             40             45

Pro Pro Arg Val Ile Arg Cys Leu Tyr Ser Arg Cys Cys Phe Gly Ile
      50             55             60

Trp Asn Leu Thr Arg Asp Gln Ala Gln Val Glu Met Gln Gly Cys Arg
      65             70             75             80

Asp Ser Asp Glu Pro Gly Cys Glu Ser Leu Ser Cys Asp Pro Ser Pro
      85             90             95

Arg Ala Arg Ala Ser Ser Gly Ser Thr Leu Phe Thr Cys Ser Cys Gly
      100            105            110

Ala Asp Phe Cys Asn Ala Asn Tyr Ser His Leu Pro Pro Leu Gly Gly
      115            120            125

Pro Gly Thr Pro Gly Pro Gln Gly Pro Gln Ala Ala Pro Gly Glu Ser
      130            135            140

Pro Trp Met Ala Leu Ala Leu Leu Gly Leu Val Leu Leu Leu Leu Leu
      145            150            155            160

Leu Leu Gly Gly Ile Val Val Ala Leu Leu Gln Arg Lys Ala Tyr Arg
      165            170            175

Val Gln Ser Gly Pro Glu Pro Glu Pro Asp Ser Gly Arg Asp Cys Ser
      180            185            190

```

- 48 -

Glu Glu Leu Pro Glu Leu Pro Gln Leu Cys Phe Ser Gln Val Ile Arg
 195 200 205

Glu Gly Gly His Ala Ala Val Trp Ala Gly Gln Leu Gln Gly Glu Leu
 210 215 220

Val Ala Ile Lys Val Phe Pro Arg Arg Ala Val Ala Gln Phe Arg Ala
 225 230 235 240

Glu Arg Ala Leu Tyr Glu Leu Pro Gly Leu Gln His Asn His Val Val
 245 250 255

Arg Phe Ile Ala Ala Gly Gln Gly Gly Pro Gly Pro Leu Pro Ser Gly
 260 265 270

Pro Leu Leu Val Leu Glu Leu His Pro Lys Gly Ser Leu Cys Gln Tyr
 275 280 285

Leu Ser Gln His Thr Ser Asp Trp Gly Ser Ser Leu Arg Met Ala Leu
 290 295 300

Ser Leu Ala Gln Gly Leu Ala Phe Leu His Glu Glu Arg Trp Gln Asp
 305 310 315 320

Gly Gln Tyr Lys Pro Gly Ile Ala His Arg Asp Leu Ser Ser Gln Asn
 325 330 335

Val Leu Ile Arg Glu Asp Gly Ser Cys Ala Ile Gly Asp Leu Gly Leu
 340 345 350

Ala Leu Val Leu Pro Gly Phe Ala Gln Pro Arg Ala Trp Ala Pro Pro
 355 360 365

Gln Pro Arg Gly Pro Ala Ala Ile Met Glu Ala Gly Thr Gln Arg Tyr
 370 375 380

Met Ala Pro Glu Leu Leu Asp Lys Ser Leu Asp Leu Gln Asp Trp Gly
 385 390 395 400

Thr Ala Leu Arg Arg Ala Asp Val Tyr Ser Leu Ala Leu Leu Leu Trp
 405 410 415

Glu Ile Leu Ser Arg Cys Pro Asp Leu Arg Pro Asp Gly Arg Pro Pro
 420 425 430

Pro Phe Gln Leu Ala Tyr Glu Ala Glu Leu Gly Ser Ala Pro Thr Thr
 435 440 445

Cys Glu Leu Trp Ala Leu Ala Val Glu Glu Arg Arg Arg Pro Asp Ile
 450 455 460

Pro Ser Ser Trp Cys Cys Phe Ala Thr Asp Pro Gly Gly Leu Arg Glu
 465 470 475 480

- 49 -

Leu Leu Glu Asp Cys Trp Asp Ala Asp Pro Glu Ala Arg Leu Thr Ala
 485 490 495
 Glu Cys Val Gln Gln Arg Leu Val Ala Leu Val His Pro Gln Glu Ala
 500 505 510
 Gln Pro Cys Pro Glu Gly Arg Pro His Ser His Pro Glu Asp Trp Pro
 515 520 525
 Pro Ala Pro Ala Pro Ala Pro Ala Leu Leu Pro Gly Ser Pro Gln Pro
 530 535 540
 Gly Ala Cys His Phe Gly Val Gln Gln Gly Leu Cys Ser Arg Asn Pro
 545 550 555 560
 Gly Ala Ala Cys Ala Ser Ser Asp Val
 565

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 6 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Tyr Met Ala Pro Glu Val
 1 5

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 17 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ACYTONGGNG CCATTTA

- 50 -

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 6 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Tyr Met Ala Pro Glu Leu
1 5

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GCAGGATGCT GGGCACTCTG

20

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GTCAGCACCA CAGGAGCAGG

20

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single

- 51 -

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CGCAGGAAGC AGTGOCCAAA

20

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ACACACAGGT OCTOCIGTTT

20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1833 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: misc feature
- (B) LOCATION: 1..1833
- (D) OTHER INFORMATION: /note= "clone 3-2"

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 61..111
- (D) OTHER INFORMATION: /note= "putative signal sequence"

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
- (B) LOCATION: 112..1779

(ix) FEATURE:

- (A) NAME/KEY: misc feature
- (B) LOCATION: 112..492
- (D) OTHER INFORMATION: /note= "extracellular domain"

- 52 -

(ix) FEATURE:

(A) NAME/KEY: misc feature

(B) LOCATION: 493..570

(D) OTHER INFORMATION: /note= "transmembrane region"

(ix) FEATURE:

(A) NAME/KEY: misc feature

(B) LOCATION: 571..1779

(D) OTHER INFORMATION: /note= "cytoplasmic domain"

(ix) FEATURE:

(A) NAME/KEY: 5'UTR

(B) LOCATION: 1..60

(ix) FEATURE:

(A) NAME/KEY: 3'UTR

(B) LOCATION: 1780..1833

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

| | |
|--|------|
| CCAGGGGCAG CIGTGCIGGC TTATGCTCTT CTCCTCTGCG TGCIGGCATC CTCAGCAAG | 60 |
| ATGCTAGGGT CTTTGGGGCT TTGGGCATTA CTTCCACAG CIGTGGAGC ACCCCAAC | 120 |
| AGGCGAACT GIGTGTCTT TGAGGCGCT GGAGTGGGG GAAGCAGAA GACACTGGG | 180 |
| GAGTGTCTAG ATACAGGCAC AGAGCTCCCG AGAGCTATCC GCCTGCTCTA CAGCGCTGC | 240 |
| TGCTTGGGA TCTGGAACT GACCAAGAC CGGGCACAGG TGGAAATGA AGGATGCGA | 300 |
| GACAGTATG AGCCAGGCTG TGAGTCCCTC CACGTGACC CAAGTCCCG AGCCACCC | 360 |
| AGCCCTGGCT CCCTCTCTT CACCTCTCC TGTGGCTG ACTTCTGCA TGGCAATTAC | 420 |
| AGCATCTGC CTCCTCCAGG GAGCCCTGG ACTCTGGCT CCAGGGTCC CCAGGCTGC | 480 |
| CCAGGTAGT CCATCTGGAT GGCACGGTG CIGCTGGGG TGTCTCTCT CCTCTGCTG | 540 |
| CTGCTGGCA GCATCTCTT GCGCTGCTA CAGCGAAGA ACTACAGAT GCGAGGTGAG | 600 |
| CCAGTGCAG AGCCAGGCT AGACTCAGG AGGACTGGA GTGTGGAGT GCAGGAGCTG | 660 |
| CTGAGCTGT GTTCTTCCA GGTATCCCG GAGGAGGTC ATGCAGTGT TTGGGCGGG | 720 |
| CAGTGCAG GAAACCTGT TGCATCAAG GCTTCCAC CGAGGTCTGT GGCCTAGTTC | 780 |
| CAAGCTGGA GAGCATTTGA CGAATTTCA GGCCTACAG AGGACACAT TGTCCGATT | 840 |
| ATCAGTCCA GCGGGGGGG TCTTGGGCG CIGCTCTCTG GCGCCCTGCT GGTACTGGAA | 900 |
| CTGCATCCA AGGCTTCCCT GTGCCTTAC TTGACCACT ACACAGTGA CTTGGGAAGT | 960 |
| TCTTGGGA TGGCACTGT CCTGGGCGG GGCCTGGCAT TTCTCCATGA GGAGGCTGG | 1020 |

- 53 -

```

CAGATGGGC AATATAAAC AGGTATTGCC CACCGAGATC TGAGCAGCCA GAATGTGCTC      1080
ATTGGGAAG ATGGATGGTG TGCCATTGGA GAACITGGGC TTGGCTTGGT GCTGCTGGC      1140
CTCCTCAGC CCCCCTGCTG GACCCCTACT CAACTACAAG GCGCAGCTGC CATCATGGAA      1200
GCCTGCAACC AGAGGTACAT GGCACCAAG CTCCTGGACA AGACCTGGA CCTACAGGT      1260
TGGGSCATGG CCGTGGAGG AGCTGATATT TACTCTTTGG CTCCTGCTCT GTGGGAGATA      1320
CTGAGCGCT GCGCAGTTT GAGGCTGAC AGCAGTCCAC CACCTTCCA ACTGGCTAT      1380
GAGGCAGAAC TGGGCATAC CCGTACCTCT GATGAGCTAT GGGCTTGGC AGTGCAGGAG      1440
AGGAGGCTC CCTACATCC ATCCACCTGG CGCTGCTTTG CCACAGAAC TGATGGGCTG      1500
AGGAGCTCC TAGAGACTG TTGGGATGA GACCCAGAAG CACGGCTGAC AGCTGAGTG      1560
GTACAGCAGC GCGTGGCTG CTCGGGCGAT CCTCAAGAGA GCGACCCCTT TCAGAGAGC      1620
TGTCAGGTG GCTGCGCAC TCCTGCGCA GAAGACTGTA CTCCAATTCC TGCGGCTAAC      1680
ATCCCTGCTT GTAGGCTCA GCGGAGTGC TGCCACTTCA GCGTTCAGCA AGCGGCTTGT      1740
TCAGGAATC CTCAGCTGC CTGTACCTT TCCTCTGCTT AAATATGAG TTTATGTGTC      1800
ATCAATGTAC ATGCCAACAT AAATATGGCG ATT                                     1833

```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 573 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Domain
- (B) LOCATION: 18..144
- (D) OTHER INFORMATION: /note= "extracellular domain"

(ix) FEATURE:

- (A) NAME/KEY: Domain
- (B) LOCATION: 145..170
- (D) OTHER INFORMATION: /note= "transmembrane region"

(ix) FEATURE:

- (A) NAME/KEY: Domain
- (B) LOCATION: 171..573
- (D) OTHER INFORMATION: /note= "cytoplasmic domain"

- 54 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Met Leu Gly Ser Leu Gly Leu Trp Ala Leu Leu Pro Thr Ala Val Glu
1 5 10 15

Ala Pro Pro Asn Arg Arg Thr Cys Val Phe Phe Glu Ala Pro Gly Val
20 25 30

Arg Gly Ser Thr Lys Thr Leu Gly Glu Leu Leu Asp Thr Gly Thr Glu
35 40 45

Leu Pro Arg Ala Ile Arg Cys Leu Tyr Ser Arg Cys Cys Phe Gly Ile
50 55 60

Trp Asn Leu Thr Gln Asp Arg Ala Gln Val Glu Met Gln Gly Cys Arg
65 70 75 80

Asp Ser Asp Glu Pro Gly Cys Glu Ser Leu His Cys Asp Pro Ser Pro
85 90 95

Arg Ala His Pro Ser Pro Gly Ser Thr Leu Phe Thr Cys Ser Cys Gly
100 105 110

Thr Asp Phe Cys Asn Ala Asn Tyr Ser His Leu Pro Pro Pro Gly Ser
115 120 125

Pro Gly Thr Pro Gly Ser Gln Gly Pro Gln Ala Ala Pro Gly Glu Ser
130 135 140

Ile Trp Met Ala Leu Val Leu Leu Gly Leu Phe Leu Leu Leu Leu Leu
145 150 155 160

Leu Leu Gly Ser Ile Ile Leu Ala Leu Leu Gln Arg Lys Asn Tyr Arg
165 170 175

Val Arg Gly Glu Pro Val Pro Glu Pro Arg Pro Asp Ser Gly Arg Asp
180 185 190

Trp Ser Val Glu Leu Gln Glu Leu Pro Glu Leu Cys Phe Ser Gln Val
195 200 205

Ile Arg Glu Gly Gly His Ala Val Val Trp Ala Gly Gln Leu Gln Gly
210 215 220

Lys Leu Val Ala Ile Lys Ala Phe Pro Pro Arg Ser Val Ala Gln Phe
225 230 235 240

Gln Ala Glu Arg Ala Leu Tyr Glu Leu Pro Gly Leu Gln His Asp His
245 250 255

Ile Val Arg Phe Ile Thr Ala Ser Arg Gly Gly Pro Gly Arg Leu Leu
260 265 270

- 55 -

Ser Gly Pro Leu Leu Val Leu Glu Leu His Pro Lys Gly Ser Leu Cys
 275 280 285
 His Tyr Leu Thr Gln Tyr Thr Ser Asp Trp Gly Ser Ser Leu Arg Met
 290 295 300
 Ala Leu Ser Leu Ala Gln Gly Leu Ala Phe Leu His Glu Glu Arg Trp
 305 310 315 320
 Gln Asn Gly Gln Tyr Lys Pro Gly Ile Ala His Arg Asp Leu Ser Ser
 325 330 335
 Gln Asn Val Leu Ile Arg Glu Asp Gly Ser Cys Ala Ile Gly Asp Leu
 340 345 350
 Gly Leu Ala Leu Val Leu Pro Gly Leu Thr Gln Pro Pro Ala Trp Thr
 355 360 365
 Pro Thr Gln Pro Gln Gly Pro Ala Ala Ile Met Glu Ala Gly Thr Gln
 370 375 380
 Arg Tyr Met Ala Pro Glu Leu Leu Asp Lys Thr Leu Asp Leu Gln Asp
 385 390 395 400
 Trp Gly Met Ala Leu Arg Arg Ala Asp Ile Tyr Ser Leu Ala Leu Leu
 405 410 415
 Leu Trp Glu Ile Leu Ser Arg Cys Pro Asp Leu Arg Pro Asp Ser Ser
 420 425 430
 Pro Pro Pro Phe Gln Leu Ala Tyr Glu Ala Glu Leu Gly Asn Thr Pro
 435 440 445
 Thr Ser Asp Glu Leu Trp Ala Leu Ala Val Gln Glu Arg Arg Arg Pro
 450 455 460
 Tyr Ile Pro Ser Thr Trp Arg Cys Phe Ala Thr Asp Pro Asp Gly Leu
 465 470 475 480
 Arg Glu Leu Leu Glu Asp Cys Trp Asp Ala Asp Pro Glu Ala Arg Leu
 485 490 495
 Thr Ala Glu Cys Val Gln Gln Arg Leu Ala Ala Leu Ala His Pro Gln
 500 505 510
 Glu Ser His Pro Phe Pro Glu Ser Cys Pro Arg Gly Cys Pro Pro Leu
 515 520 525
 Cys Pro Glu Asp Cys Thr Ser Ile Pro Ala Pro Thr Ile Leu Pro Cys
 530 535 540
 Arg Pro Gln Arg Ser Ala Cys His Phe Ser Val Gln Gln Gly Pro Cys
 545 550 555 560

- 56 -

Ser Arg Asn Pro Gln Pro Ala Cys Thr Leu Ser Pro Val
 565 570

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 900 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: exon
- (B) LOCATION: 1..109
- (D) OTHER INFORMATION: /note= "exon 1"

(ix) FEATURE:

- (A) NAME/KEY: intron
- (B) LOCATION: 110..413
- (D) OTHER INFORMATION: /note= "intron A"

(ix) FEATURE:

- (A) NAME/KEY: exon
- (B) LOCATION: 414..596
- (D) OTHER INFORMATION: /note= "exon 2"

(ix) FEATURE:

- (A) NAME/KEY: intron
- (B) LOCATION: 597..834
- (D) OTHER INFORMATION: /note= "intron B"

(ix) FEATURE:

- (A) NAME/KEY: exon
- (B) LOCATION: 835..900
- (D) OTHER INFORMATION: /note= "exon 3 (partial)"

(ix) FEATURE:

- (A) NAME/KEY: misc feature
- (B) LOCATION: 597
- (D) OTHER INFORMATION: /note= "nucleotide changed to A in patient T.A."

(xd) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```

CCAGGGGCAG CIGTCTGGC TTATGCTCTT CTCCTTCCTC TCTGGCATC CTCAGCAAG      60
ATGCTAGGGT CTTTGGGGCT TTGGGCATTA CTTCCACAG CIGTGAAGG TAAGTGTCTA      120
CAGGGAGGGG AAGGGTCTCT CCATCATCC AGCAAGGGA AGGGGGCTT GAAGCAAGAG      180

```

- 57 -

```

CCACCCCCTT GGAAGATGG TGAGTGGCT GGGTGTAA GGGTGAAGCA TAGAGCCATG      240
TGTCGCCATG GCAGGGCTCA GGTTCCAGGC CTCCTCTGAC CCTGCTTCTT CCTGTGGCTT      300
TACCATACAG ACGCTGGGAT GGGGAACATG TTTTGTCTAT TCTTTTGGCC AGTTTTTTGC      360
CTCTGCATTC ACTTCCACTT TGAATCTTTT CCTTTTCCCA CCTTGGGCTT CAGCAACCCC      420
AAACAGGCGA AACTGTGTGT TCTTTGAGGC CCTTGGAGTG CCGGGAAGCA CAAAGACACT      480
GGGAGAGCTG CTAGATACAG GCACAGAGCT CCCCAGAGCT ATCCGCTGCC TCTACAGCCG      540
CTCTGCTTTT GGGATCTGGA AACTGAACCA AGAOCGGGCA CAGGTGGAAA TGCAAGGTGA      600
ATGGCAAAGT ATATGGCAGG TGATGGCTAG GGTGGGAGAC AGACACATCC TGGGGTGTGG      660
GTGGCAACCA AGGGGTAAGG GGAGAANTAG AACATCTGGT GGGAAAGAAA AGCCCATGAG      720
AGCTGGAAGG GAGGCTCTG ATAGAGAAGG GATTTAOCCT CTTTTCACAC ACCCCATTGT      780
GCTTCTTCTT TTTGGGGGGG TTTCTCTCTT CTTCCTTAA TCCATCCCA TCAGGATGCC      840
GAGACAGTGA TGAGCCAGGC TGTGAGTCC TCCACTGTGA CCAAGTCC CGAGCCACC      900

```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: misc feature
- (B) LOCATION: 1..20
- (D) OTHER INFORMATION: /note= "5' sense PCR primer 1s from human AMH receptor cDNA (nucleotides 36-55 of SEQ ID NO: 12)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TCCTCTCTCT CCATCTTCCA

20

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid

- 58 -

- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: misc feature
- (B) LOCATION: 1..21
- (D) OTHER INFORMATION: /note= "3' antisense PCR primer 2a from human AMH receptor cDNA (complement of nucleotides 410-430 of SEQ ID NO: 12)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GCAGATGGCT GTAATGGCA T

21

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: misc feature
- (B) LOCATION: 1..20
- (D) OTHER INFORMATION: /note= "3' antisense PCR primer 3a from human AMH receptor cDNA (complement of nucleotides 504-523 of SEQ ID NO: 12)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ACAGCCCCAG CAGCACCAGT

20

58/1

| | | |
|---|--------------|-------------------------------|
| Applicant's or agent's file reference number | B174 CIP PCT | International application No. |
|---|--------------|-------------------------------|

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

| | |
|--|---|
| A. The indications made below relate to the microorganism referred to in the description on page <u>27</u> , lines <u>16-19</u> | |
| B. IDENTIFICATION OF DEPOSIT <u>E. coli</u> . Further deposits are identified on an additional sheet <input checked="" type="checkbox"/> | |
| Name of depositary institution <u>MC1061/P3/pB210</u> <u>American Type Culture Collection</u> | |
| Address of depositary institution (including postal code and country) <u>12301 Parklawn Drive</u> <u>Rockville, Maryland 20852</u> <u>United States of America</u> | |
| Date of deposit <u>16 December 1993</u> <u>(16.12.93)</u> | Accession Number <u>69520</u> |
| C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input checked="" type="checkbox"/> In respect of the designation of the EPO, samples of the deposited microorganisms will be made available until the publication of the mention of the grant of the European patent or until the date on which the application is refused or withdrawn or is deemed to be withdrawn, as provided in Rule 28(3) of the Implementing Regulations under the EPC only by the issue of a sample to an expert nominated by the requester (Rule 28(4) EPC). | |
| D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States) <u>EPO</u> | |
| E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit") | |
| For receiving Office use only <input checked="" type="checkbox"/> This sheet was received with the international application Authorized officer <u>[Signature]</u> <u>STEVEN R. BODIS</u> <u>INTERNATIONAL BUREAU</u> | For International Bureau use only <input type="checkbox"/> This sheet was received by the International Bureau on: Authorized officer |

58/2

Agent's file
reference number: B174 CIP PCT

Form PCT/RO/134

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

B. IDENTIFICATION OF DEPOSIT

E. coli, MC1061/P3/pB210, Accession Number 69520

Continuation of Box C.

In respect of the designation of Finland, until the application has been laid open to public inspection by the Finnish Patent Office, or has been finally decided upon by the Finnish Patent Office without having been laid open to public inspection, samples of the deposited microorganisms will be made available only to an expert in the art.

Continuation of Box D.

Finland

58/3

| | | |
|--|--------------|-------------------------------|
| Applicant or agent's file reference number | B174 CIP PCT | International application No. |
|--|--------------|-------------------------------|

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

| | | | |
|--|---|---|---|
| A. The indications made below relate to the microorganism referred to in the description on page <u>27</u> . line s <u>19-22</u> | | | |
| B. IDENTIFICATION OF DEPOSIT <u>E. coli</u> , Further deposits are identified on an additional sheet <input checked="" type="checkbox"/> | | | |
| Name of depositary institution <u>MC1061/P3/pH1</u> <u>American Type Culture Collection</u> | | | |
| Address of depositary institution (including postal code and country) <u>12301 Parklawn Drive</u> <u>Rockville, Maryland 20852</u> <u>United States of America</u> | | | |
| Date of deposit <u>16 December 1993</u> <u>(16.12.93)</u> | Accession Number <u>69521</u> | | |
| C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input checked="" type="checkbox"/> | | | |
| In respect of the designation of the EPO, samples of the deposited microorganisms will be made available until the publication of the mention of the grant of the European patent or until the date on which the application is refused or withdrawn or is deemed to be withdrawn, as provided in Rule 28(3) of the Implementing Regulations under the EPC only by the issue of a sample to an expert nominated by the requester (Rule 28(4) EPC). | | | |
| D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States) <u>EPO</u> | | | |
| E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., 'Accession Number of Deposits') | | | |
| <table border="1"> <tr> <td> For receiving Office use only <input checked="" type="checkbox"/> This sheet was received with the international application Authorized officer <u>[Signature]</u> <u>INTERNATIONAL DIVISION</u> </td> <td> For International Bureau use only <input type="checkbox"/> This sheet was received by the International Bureau on: Authorized officer </td> </tr> </table> | | For receiving Office use only <input checked="" type="checkbox"/> This sheet was received with the international application Authorized officer <u>[Signature]</u> <u>INTERNATIONAL DIVISION</u> | For International Bureau use only <input type="checkbox"/> This sheet was received by the International Bureau on: Authorized officer |
| For receiving Office use only <input checked="" type="checkbox"/> This sheet was received with the international application Authorized officer <u>[Signature]</u> <u>INTERNATIONAL DIVISION</u> | For International Bureau use only <input type="checkbox"/> This sheet was received by the International Bureau on: Authorized officer | | |

58/4

Agent's file
reference number: B174 CIP PCT

Form PCT/RO/134

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

B. IDENTIFICATION OF DEPOSIT

E. coli, MC1061/P3/pH1, Accession Number 69521

Continuation of Box C.

In respect of the designation of Finland, until the application has been laid open to public inspection by the Finnish Patent Office, or has been finally decided upon by the Finnish Patent Office without having been laid open to public inspection, samples of the deposited microorganisms will be made available only to an expert in the art.

Continuation of Box D.

Finland

58/5

| | | |
|--|--------------|-------------------------------|
| Applicant's or agent's file reference number | B174 CIP PCT | International application No. |
|--|--------------|-------------------------------|

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

| | |
|--|------------------------|
| A. The indications made below relate to the microorganism referred to in the description on page <u>34</u> , line <u>5</u> <u>23-26</u> | |
| B. IDENTIFICATION OF DEPOSIT | Plasmid |
| Further deposits are identified on an additional sheet <input type="checkbox"/> | |
| Name of depositary institution <u>hAMHR3-2</u> | |
| <u>American Type Culture Collection</u> | |
| Address of depositary institution (including postal code and country) <u>12301 Parklawn Drive</u> <u>Rockville, Maryland 20852</u> <u>United States of America</u> | |
| Date of deposit <u>13 December 1994</u> <u>(13.12.94)</u> | Accession Number _____ |
| C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input checked="" type="checkbox"/> | |
| In respect of the designation of the EPO, samples of the deposited microorganisms will be made available until the publication of the mention of the grant of the European patent or until the date on which the application is refused or withdrawn or is deemed to be withdrawn, as provided in Rule 28(3) of the Implementing Regulations under the EPC only by the issue of a sample to an expert nominated by the requester (Rule 28(4) EPC). | |
| D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States) | |
| <u>EPO</u> | |
| E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) | |
| The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit") | |
| <u>Accession Number of Deposit</u> | |

| | |
|--|--|
| For receiving Office use only | For International Bureau use only |
| <input checked="" type="checkbox"/> This sheet was received with the international application | <input type="checkbox"/> This sheet was received by the International Bureau on: |
| Authorized officer <u>[Signature]</u> <u>WILLIAM B. FARRIS</u> <u>INTERNATIONAL DIVISION</u> | Authorized officer _____ |

58/6

Agent's file
reference number: B174 CIP PCT

Form PCT/RO/134

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

B. IDENTIFICATION OF DEPOSIT

Plasmid hAMHR3-2, Accession Number _____

Continuation of Box C.

In respect of the designation of Finland, until the application has been laid open to public inspection by the Finnish Patent Office, or has been finally decided upon by the Finnish Patent Office without having been laid open to public inspection, samples of the deposited microorganisms will be made available only to an expert in the art.

Continuation of Box D.

Finland

- 59 -

We claim:

1. An isolated DNA sequence comprising a DNA sequence encoding the mature AMH receptor polypeptide selected from the group consisting of

- (a) nucleotides 113 to 1585 of SEQ ID NO: 1;
- (b) nucleotides 110 to 1765 of SEQ ID NO: 2;
- (c) nucleotides 112 to 1779 of SEQ ID NO: 12;
- (d) DNA sequences that hybridize to any of the DNA sequences of (a)-(c) and which encode a biologically or antigenically active AMH receptor polypeptide; and
- (e) DNA sequences that are degenerate to any of the foregoing DNA sequences.

2. An isolated DNA sequence according to claim 1 encoding the extracellular domain of the AMH receptor polypeptide selected from the group consisting of

- (a) nucleotides 113 to 310 of SEQ ID NO: 1;
- (b) nucleotides 110 to 490 of SEQ ID NO: 2;
- (c) nucleotides 112 to 492 of SEQ ID NO: 12;
- (d) DNA sequences that hybridize to any of the DNA sequences of (a)-(c) and which encode a biologically or antigenically active AMH receptor polypeptide; and
- (e) DNA sequences that are degenerate to any of the foregoing DNA sequences.

3. An AMH receptor polypeptide encoded by the isolated DNA of claim 1.

4. An AMH receptor polypeptide according to claim 3 comprising an amino acid sequence corresponding to the mature AMH receptor polypeptide selected from the group consisting of

- (a) amino acid residues 18 to 508 of SEQ ID NO: 3;

- 60 -

- (b) amino acid residues 18 to 569 of SEQ ID NO: 4;
and
- (c) amino acid residues 18 to 573 of SEQ ID NO: 13.

5. An AMH receptor polypeptide according to claim 3 comprising an amino acid sequence corresponding to the extracellular domain the mature AMH receptor polypeptide selected from the group consisting of

- (a) amino acid residues 18 to 83 of SEQ ID NO: 3;
- (b) amino acid residues 18 to 144 of SEQ ID NO: 4;
and
- (c) amino acid residues 18 to 144 of SEQ ID NO: 13.

6. An antibody or antibody homolog which specifically recognizes an AMH receptor polypeptide according to claim 3.

7. An antibody or antibody homolog according to claim 6 which recognizes the extracellular domain of the AMH receptor polypeptide.

8. An antibody or antibody homolog according to claim 6 which recognizes the AMH receptor in its native conformation on the surface of cells.

9. An antibody or antibody homolog according to claim 6 which is conjugated to a further therapeutic agent selected from the group consisting of a toxin and a radionuclide.

10. A method of detecting the presence of an AMH receptor in a biological sample comprising the steps of

- (a) contacting an antibody according to claim 6 with a biological sample suspected of containing an AMH receptor; and

- 61 -

(b) detecting immune complex formation between said antibody and a component of said biological sample, wherein said immune complex formation is indicative of the presence of an AMH receptor in said biological sample.

11. A method for detecting AMH ligand in a biological sample comprising the steps of

(a) contacting an AMH receptor polypeptide according to claim 3 with a biological sample suspected of containing an AMH ligand; and

(b) detecting binding between said polypeptide and a component of said biological sample, wherein said binding is indicative of the presence of an AMH ligand in said biological sample.

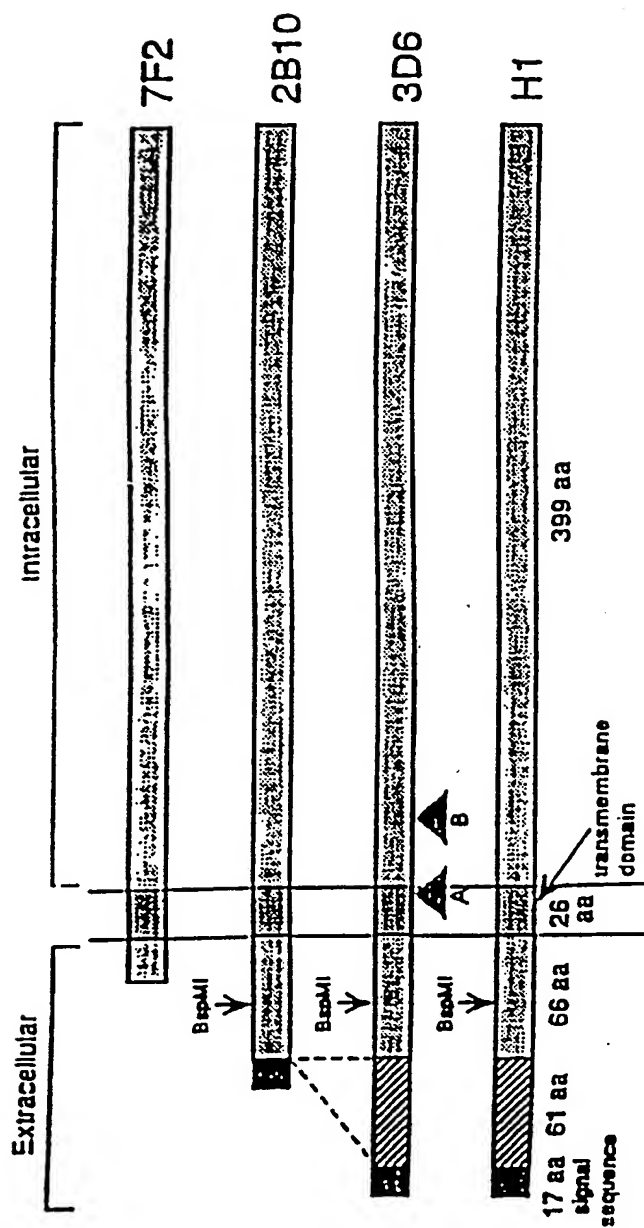


FIG. 1A

2/14

[illegible]

FIG. 1B

3/14

1201 ACAGAGGTACATGGCGCCAGAGCTCTTGGACAAGTCTCTGGACCTACAGGACTGGGCACTGCCCTCCGGCGAGCCGACGCTCTACTCTCTGGCCCTGCTC 1300
Q R Y M A P E L L D K S L D L Q D W G T A L R R A D V Y S U A L L

1301 CTGTGGGAGATCCTGAGCGCTGCCCGGATTGAGGCGCTGACGCGAGACCACCCCTTCCAACTGGCCTATGAAGCAGAACTGGGCGAGCGCCCCACCA 1400
L W E I L S R C P D L R P D G R P P F Q L A Y E A E L G S A P T T

1401 CCTGTGAGCTGTGGCCCTGGCAGTAGAGGAGGCGCCCTGACATCCCATCCTCTGGTGTCTTTGCCACAGACCCCGGGGCTCAGGGAGCT 1500
C E L W A L A V E E R R R P D I P S S W C C F A T D P G G L R E L

1501 GCTGGAAGACTGTGGACGCGAGACCCGGAAGCGCGCTGACGGCGGAGTGTGTCCAGCAGCGCCTGGTGGCCCTGGTTCACTCTCAGGAGCGCCAGCCC 1600
L E D C W D A D P E A R L T A E C V Q Q R L V A L V H P Q E A Q P

1601 TGCCCAGAGGGCGTCCACACAGCCACCCAGAGACTGGCCCCCTGCTCCTGCCCTGCCCTCTCCTCCCGGCGAGCCACAGCCGCGTGCCTGCC 1700
C P E G R P H S H P E D W P P A P A P A L L P G S P Q P G A C H

1701 ACTTCGGTGTTCAGCAAGCCCTTTGCTCCAGGAACCCCGGAGCTGCCCTGTGCCAGTTCTGACGTCTAAATAAGCAGTTTGTGTGTAATCTACCTGTAAAC 1800
F G V Q Q G L C S R N P G A A C A S S D V

1801 GTAAACATGGCACTGGTATACCTGTCTGCTCTCCACTGTTTCCCACTCTCTAGGCAGGAAGCTGGAATTTGAGCCCTATGTGTGTG 1900

1901 TGTGTGAGACAGGCCCTGAAGACCAAGCACCATTGCAGAAGCCCCAGAAACCCAGCATCTTGCACCTCAGAGTCTGCCCGAGACCCCTGCTAACCTCCAGG 2000

2001 TGGTCCCAGCCCTTCCCTCAGGCAAACTCCAGGAGAAATTTCTCTCTCCTCAGGACCAAGGGTTACCTGTATCTGATACACAGGCGCATAAACCTTCA 2100
End of 3D6.

2101 CAGAGTCCCTTGCCAGCAAACTGGGAGGAATTTGCAAAATTTTCACTCAACAAACCTTCCACAGGGCTCCCCCTTCTTTGTCTCGAGGAGAAAGG 2200

2201 GAGGTGGGTAAACAGACTCCCTTAAACCTAGGGAGTCCAAACTGACACGCACTCAGCCCTCTGCTCTGCTAGCCCGCCCGCTGGCCCTGCCAG 2300

2301 CTGTATTCTTTCAATTCAACCATGTGACCTTGCTCTTCCCTGAGCGCTCTCTGTCTGTCTCCCTCCCGTTTGTGACAGATGCCCTGTCTCCCAATAAACCTTA 2400
Poly A signal

2401 TCACTCTG 2408

FIG. 1C

4/14

[illegible]

FIG. 1D

RECTIFIED SHEET (RULE 91)
ISA/EP

5/14

FIG. 2A

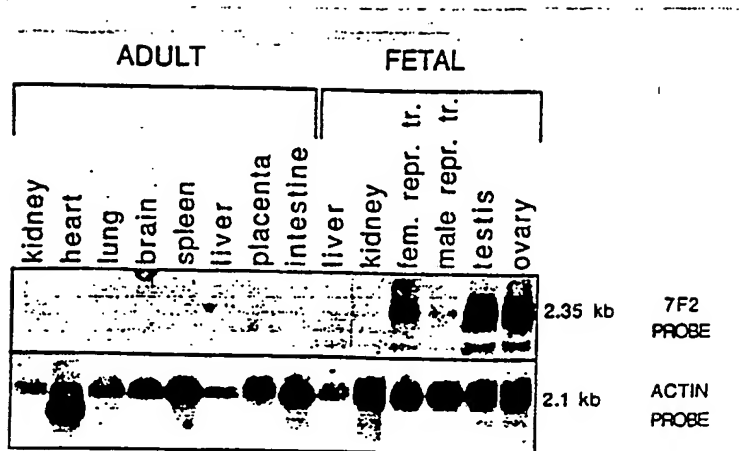
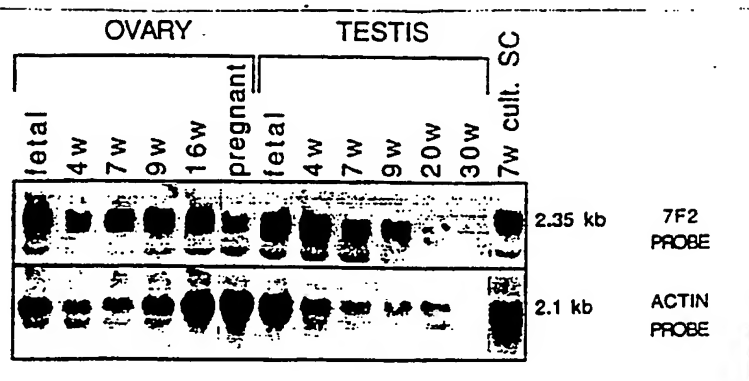


FIG. 2B



6/14

FIG. 2C



FIG. 2D

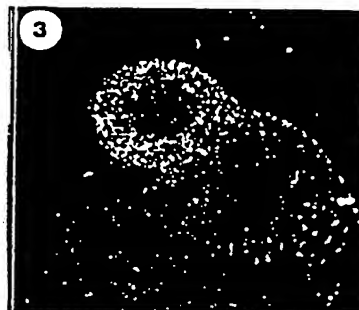


FIG. 2E

7/14

FIG. 3A

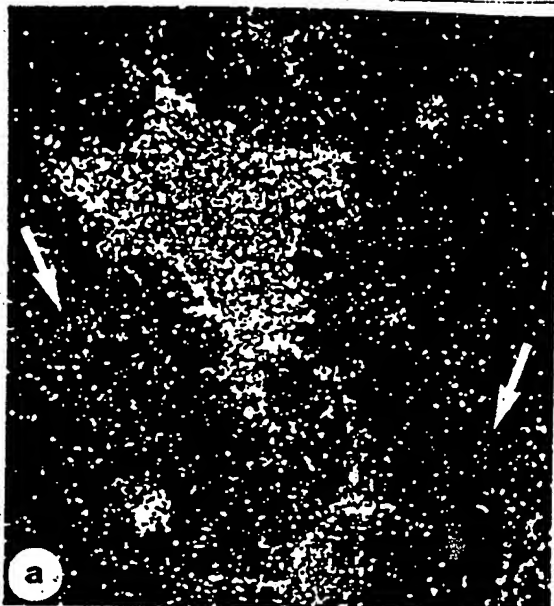


FIG. 3B

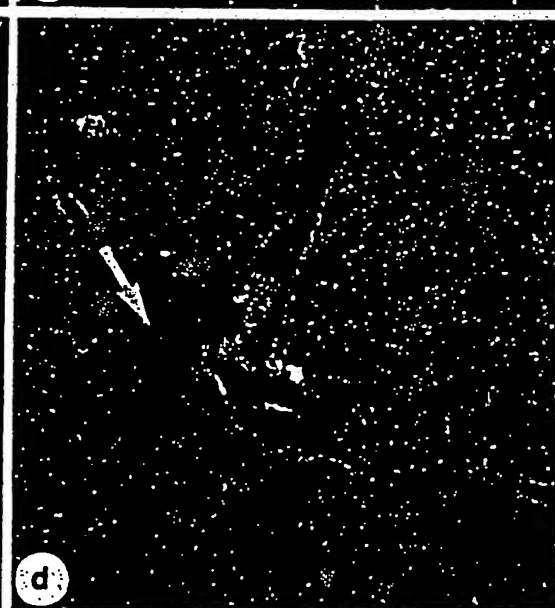
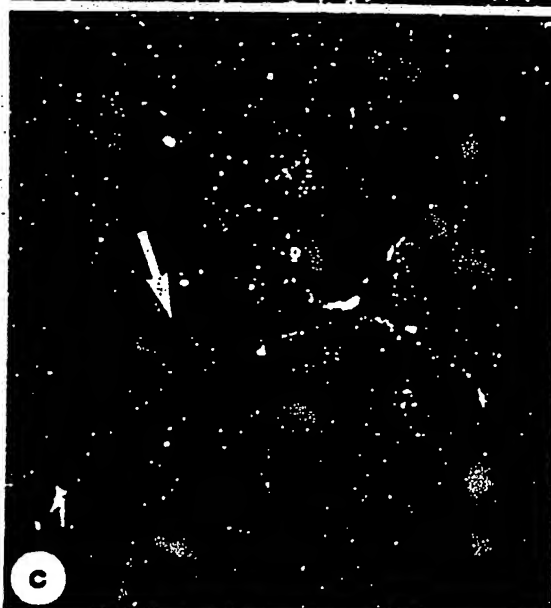


FIG. 3C

FIG. 3D

8/14

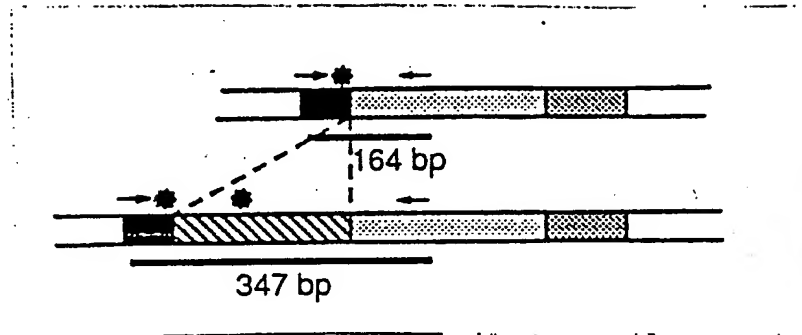


FIG. 4A

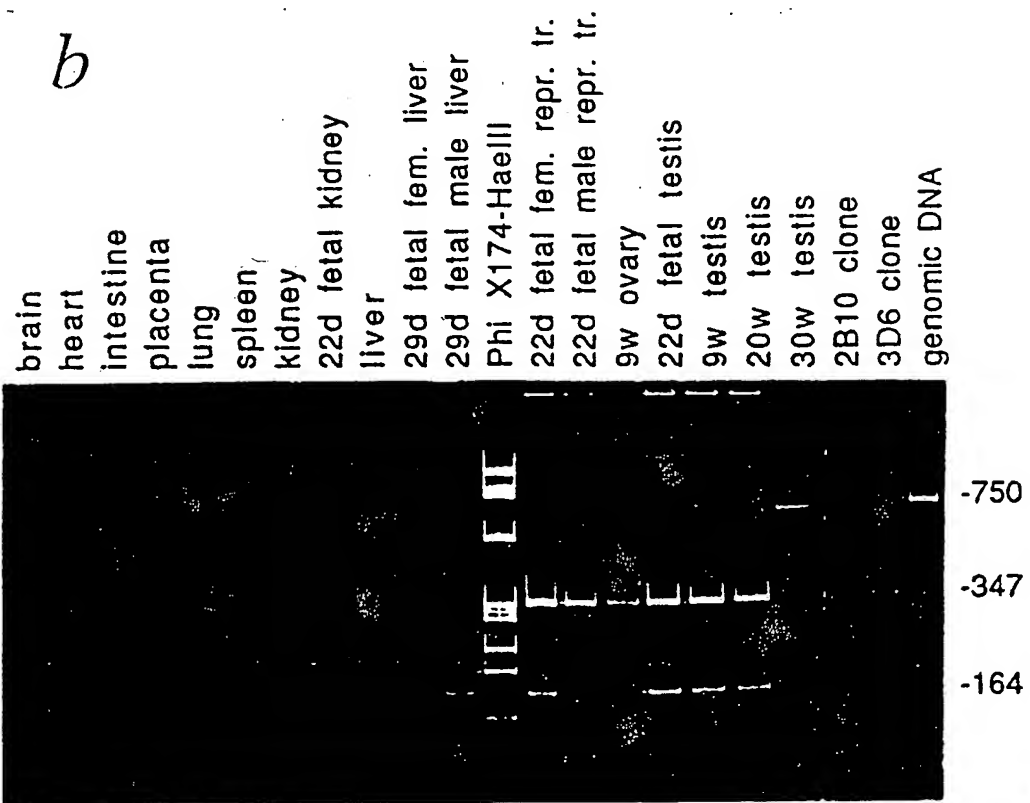


FIG. 4B

9/14

```

1 MLGSLGLWALLPTAVEAPPNRRTC VFF EAPGVRGSTKTLGELLDTGTLP 50
  |||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
1 MLGTLGLWALLPAAVQAPPNRRTC VFF EAPGVRGSTKTLGELLDAGPGPP 50
  |||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
51 RAIRCLYSRCCFGIWNLTQDRAQVEMQGC RDSDEPGCESLHCDPSRAHP 100
  |||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
51 RVIRCLYSRCCFGIWNLTRDQAQVEMQGC RDSDEPGCESLSCDPSPRARA 100
  |||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
101 SPGSTLFTCS CGTDFCNANYSHLPPPGSPGTPGSQGPQAAPGESIWMA LV 150
  |||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
101 SSGSTLFTCS CGADFCNANYSHLPPLGGPGTPGPGQGPQAAPGESPWMALA 150
  |||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
151 LLGLFLLLLLLLGSII LALLQKNYRVRGEPVPEPRPDSGRDWSVELQEL 200
  |||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
151 LLGLVLLLLLLLGGIVVALLQKAYRVQSG..PEPEPDSGRDCSEELPEL 198
  |||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
201 PELCFSQVIREGGHAAVWAGQLQGLVAIKAFFPR SVAQFOAERALYELP 250
  |||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
199 PQLCFSQVIREGGHAAVWAGQLQGLVAIKVFPRRAVAQFRAERALYELP 248
  |||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
251 GLQHDHIVRFITASRGGPGRLLSGPLLVL ELHPKGS LCHYLTOYTS DWGS 300
  |||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
249 GLQHNHVRFIAAGQGGPGPLPSGPLLVL ELHPKGS LCOYLSQHTS DWGS 298
  |||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
301 SLRMALSLAQGLAFLHEERWONGQYKPGIAHRDLSSQ NVLIREDGSCAIG 350
  |||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
299 SLRMALSLAQGLAFLHEERWODGQYKPGIAHRDLSSQ NVLIREDGSCAIG 348
  |||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
351 DLGLALVLPGLTQPPAWTPTQPGPAAIMEAGTORYMAPELLDKTLDLQD 400
  |||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
349 DLGLALVLPGFAQPRAWAPPQPRGPAAIMEAGTORYMAPELLDKSLDLQD 398
  |||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
401 WGMALRRADIYSLALLLWEILSRCPDLRPDSSPPPFQLAYEAE LGNTPTS 450
  |||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
399 WGTALRRADVYSLALLLWEILSRCPDLRPDGRPPPFQLAYEAE LGSAPTT 448
  |||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
451 DELWALAVQERRRPYIPSTWRCFATDPDGLRELLED CWDADPEARLTAEC 500
  |||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
449 CELWALAVEERRRPDIPSSWCCFATDPGGLRELLED CWDADPEARLTAEC 498
  |||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
501 VQORLAALAHPOESHFPFESCPRGCPPLCPEDCTSI PAPTILPCRQPSA 550
  |||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
499 VQORLVALVHPQEAQPCPEGRPHSHPEDWP..PAPAPAPALLPGSPQPGA 546
  |||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
551 CHFSVQQGP CSRNPQACTLSPV 573
  |||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
547 CHFGVQQGL CSRNPGAACASSDV 569
  |||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:

```

FIG. 5

10/14

[illegible]

FIG. 6

[illegible]

FIG. 7

12/14

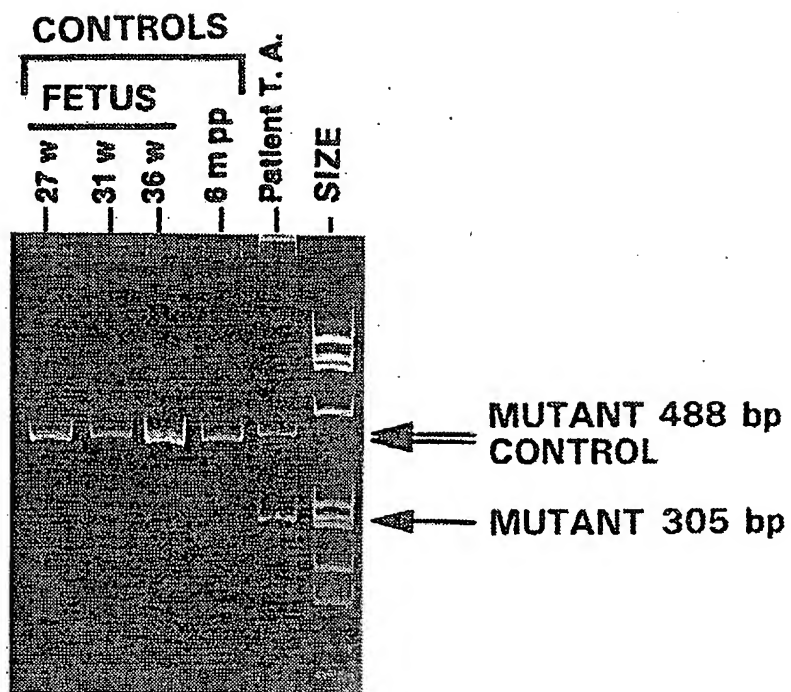
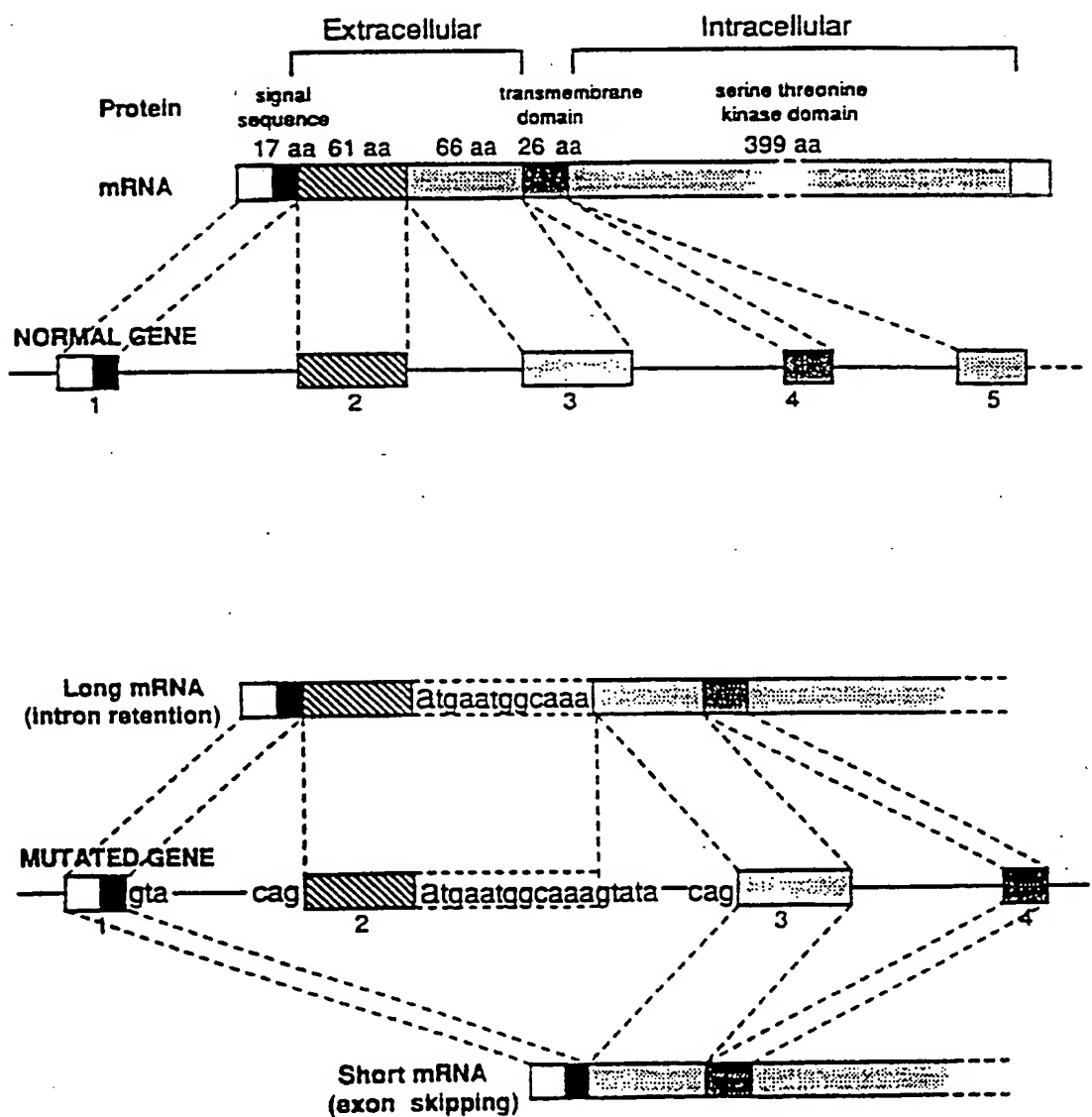


FIG. 8

13/14

FIG. 9



14/14

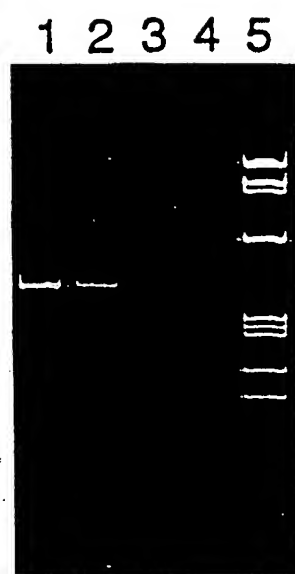


FIG. 10

| | | |
|---|--------------|-------------------------------|
| Applicant's or agent's file reference number | B174 CIP PCT | International application No. |
|---|--------------|-------------------------------|

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

| | |
|---|---|
| A. The indications made below relate to the microorganism referred to in the description on page <u>34</u> , line 6 <u>23-26</u> | |
| B. IDENTIFICATION OF DEPOSIT <u>plasmid</u> Further deposits are identified on an additional sheet <input type="checkbox"/> | |
| Name of depository institution <u>hAMHR3-2</u> <u>American Type Culture Collection</u> | |
| Address of depository institution (including postal code and country) <u>12301 Parklawn Drive</u> <u>Rockville, Maryland 20852</u> <u>United States of America</u> | |
| Date of deposit <u>13 December 1994</u> <u>(13.12.94)</u> | Accession Number <u>69719</u> |
| C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/> | |
| | |
| D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States) | |
| | |
| E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) | |
| The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit") | |
| | |
| For receiving Office use only <input type="checkbox"/> This sheet was received with the international application Authorized officer | For International Bureau use only <input type="checkbox"/> This sheet was received by the International Bureau on: <u>27 JANUARY 1995 (27.01.95)</u> Authorized officer <u>E. Moyse</u> |

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | | |
|---|--|----|---|
| (51) International Patent Classification ⁶ : C07K 14/71, 14/72, 16/28, G01N 33/74 | | A3 | (11) International Publication Number: WO 95/16709 |
| | | | (43) International Publication Date: 22 June 1995 (22.06.95) |
| (21) International Application Number: PCT/US94/14643 | | | (74) Agents: HALEY, James, F., Jr. et al.; Fish & Neave, 1251 Avenue of the Americas, New York, NY 10020-1104 (US). |
| (22) International Filing Date: 13 December 1994 (13.12.94) | | | |
| (30) Priority Data: 08/166,333 13 December 1993 (13.12.93) US 08/173,512 23 December 1993 (23.12.93) US | | | (81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ). |
| (60) Parent Applications or Grants (63) Related by Continuation US 08/166,333 (CIP) Filed on 13 December 1993 (13.12.93) US 08/173,512 (CIP) Filed on 23 December 1993 (23.12.93) | | | Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> <i>With an indication in relation to a deposited microorganism furnished under Rule 13^{bis} separately from the description.</i> <i>Date of receipt by the International Bureau:</i> 27 January 1995 (27.01.95) |
| (71) Applicants (for all designated States except US): BIOGEN, INC. [US/US]; 14 Cambridge Center, Cambridge, MA 02142 (US). INSERM (U.293) [FR/FR]; 1, rue Maurice-Arnoux, F-29120 Montrouge (FR). | | | (88) Date of publication of the international search report: 10 August 1995 (10.08.95) |
| (72) Inventors; and (75) Inventors/Applicants (for US only): CATE, Richard, L. [US/US]; 14 Cambridge Center, Cambridge, MA 02142 (US). JOSSO, Nathalie [FR/FR]; INSERM (U.293), 1, rue Maurice-Arnoux, F-29120 Montrouge (FR). | | | |
| (54) Title: ANTI-MULLERIAN HORMONE RECEPTOR POLYPEPTIDES AND ANTIBODIES THERETO | | | |
| (57) Abstract <p>This invention relates to polypeptides displaying the activity of anti-Mullerian hormone (AMH) receptor, also known as Mullerian inhibiting substance (MIS) receptors, and antibodies to those polypeptides. More particularly, this invention relates to such AMH receptor polypeptides and antibodies, processes for producing those polypeptides and antibodies and methods for using them in the treatment of cancer and tumors of tissues associated with expression of the anti-Mullerian hormone receptor.</p> | | | |

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | |
|----|--------------------------|----|---------------------------------------|----|--------------------------|
| AT | Austria | GB | United Kingdom | MR | Mauritania |
| AU | Australia | GE | Georgia | MW | Malawi |
| BB | Barbados | GN | Guinea | NE | Niger |
| BE | Belgium | GR | Greece | NL | Netherlands |
| BF | Burkina Faso | HU | Hungary | NO | Norway |
| BG | Bulgaria | IE | Ireland | NZ | New Zealand |
| BJ | Benin | IT | Italy | PL | Poland |
| BR | Brazil | JP | Japan | PT | Portugal |
| BY | Belarus | KE | Kenya | RO | Romania |
| CA | Canada | KG | Kyrgyzstan | RU | Russian Federation |
| CF | Central African Republic | KP | Democratic People's Republic of Korea | SD | Sudan |
| CG | Congo | KR | Republic of Korea | SE | Sweden |
| CH | Switzerland | KZ | Kazakhstan | SI | Slovenia |
| CI | Côte d'Ivoire | LI | Liechtenstein | SK | Slovakia |
| CM | Cameroon | LK | Sri Lanka | SN | Senegal |
| CN | China | LU | Luxembourg | TD | Chad |
| CS | Czechoslovakia | LV | Latvia | TC | Togo |
| CZ | Czech Republic | MC | Monaco | TJ | Tajikistan |
| DE | Germany | MD | Republic of Moldova | TT | Trinidad and Tobago |
| DK | Denmark | MG | Madagascar | UA | Ukraine |
| ES | Spain | ML | Mali | US | United States of America |
| FI | Finland | MN | Mongolia | UZ | Uzbekistan |
| FR | France | | | VN | Viet Nam |
| GA | Gabon | | | | |

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US/94/14643

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07K14/71 C07K14/72 C07K16/28 G01N33/74

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| X | GenBank database entry, submitted 27-04-93 Accession number X71916 BAARENDS, W.M. et al.: 'A novel member of the transmembrane serine/threonine kinase receptor family ...', see the abstract & DEVELOPMENT, vol. 120, no. 1, 1994 pages 189-197, --- | 1-11 |
| A | WO, A, 93 19177 (THE GENERAL HOSPITAL CORP.) 30 September 1993 * claims; p. 1-10 * --- -/-- | 1-11 |

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *I* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- * & * document member of the same patent family

Date of the actual completion of the international search

9 June 1995

Date of mailing of the international search report

20.06.95

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (- 31-70) 340-2040, Tx. 31 651 epo nl,
Fax (- 31-70) 340-3016

Authorized officer

Hermann, R

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 94/14643

| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT | | |
|--|---|-----------------------|
| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| A | CHEMICAL ABSTRACTS, vol. 120, no. 11, 14 March 1994 Columbus, Ohio, US; abstract no. 128396, HE, W.W. ET AL. 'Developmental expression of four novel serine/threonine kinase receptors homologous to the activin/transforming growth factor beta type II receptor family' see abstract & DEV.DYN., vol. 196,no. 2, 1993 pages 133-142, --- | 1-11 |
| A | CHEMICAL ABSTRACTS, vol. 120, no. 5, 31 January 1994 Columbus, Ohio, US; abstract no. 46292, CATLIN, E. ET AL. 'Identification of a receptor for human Mullerian inhibiting substance' see abstract & ENDOCRINOLOGY, vol. 133,no. 6, 1993 pages 3007-30013, --- | 1-11 |
| P,X | CHEMICAL ABSTRACTS, vol. 121, no. 1, 4 July 1994 Columbus, Ohio, US; abstract no. 1123, GROOTEGOE, J.A. ET AL. 'Welcome to the family: the anti-mullerian hormone receptor' see abstract & MOL. CELL. ENDOCRINOL., vol. 100,no. 1-2, 1994 pages 29-34, --- | 1-11 |
| P,X | CHEMICAL ABSTRACTS, vol. 121, no. 19, 7 November 1994 Columbus, Ohio, US; abstract no. 222195, DI CLEMENTE, N. ET AL. 'Cloning, expression and alternative splicing of the receptor for anti-mullerian hormone' see abstract & MOL. ENDOCRINOL., vol. 8,no. 8, 1994 pages 1006-1020, ----- | 1-11 |

INTERNATIONAL SEARCH REPORT

information on patent family members

| | |
|-------------------------|--|
| Internal Application No | |
|-------------------------|--|

PCT/US 94/14643

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---------------------|----------------------------|---------------------|
| WO-A-9319177 | 30-09-93 | AU-B- 3920693 | 21-10-93 |
| ----- | | | |